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Inheritance in parthenogenesis and in sexual reproduction in a cladoceran.

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For some years we have been making studies with Cladocera on the occurrence of mutations and their inheritance in parthenogenesis. Four mutant characters have been studied in *Daphnia longispina*. The inheritance is complete, since every parthenogenetic young from a mutant individual genetically possesses the mutant character (except, of course, for the occasional interference of another mutation). This is in accord with expectation, in view of the absence of chromatic reduction and segregation in the maturation of the parthenogenetic (diploid) eggs in Cladocera.

It was of further interest to obtain, if possible, sexual reproduction and hybrid offspring between mutant clones and the "wild" type. Sexual eggs (haploid and requiring fertilization) are frequently obtainable, and males may usually be obtained when desired, but it is not easy to obtain, *simultaneously*, ovarian sexual eggs and functional males from just the stocks desired. When sexual eggs and active males are obtained at the same time, fertilization frequently does not occur, and the fertilized eggs

when obtained give exceedingly poor hatches, so that many fruitless attempts preceded the obtaining of the desired hybrids.

Forty hybrids have been or are now being studied and some data obtained on the inheritance of the two mutant characters involved in the stock used in the crosses. The characters involved are sex-intergradedness and "excavated" head. Twenty-nine of these hybrids are from wild type ♀ × mutant ♂, and 11 are from the reciprocal cross. Nineteen of these hybrids genetically possess the mutant character, excavated head, and 19 show sex intergradedness. It seems probable, therefore, that each of these characters is a dominant mendelian character and is heterozygous in the mutant clones used in the crosses. Further breeding and study will be necessary before the full extent of the inheritance of these characters is determined.

The absence of both these characters in all except the mutant laboratory strains, in much wild stock examined, and in sexual offspring from wild type parents, their presence and heritability in parthenogenesis in the mutant strains, and in sexual offspring from these strains, indicate that these characters are definitely heritable and behave in bi-parental inheritance like characters in bi-parental inheritance in other organisms.

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A method for preserving and counterstaining vitally-stained cells.*

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Recently, in the course of some experiments on the viability of monocytes,[1] it became necessary to preserve, for further study, cells which had been vitally stained with neutral red. The cells

* Work done under a grant to Professor George A. Baitsell from the National Tuberculosis Association, Medical Research Committee.

1 Sabin, F. R., Doan, C. A., and Cunningham, R. S., Contributions to Embryology No. 82, Carnegie Inst. of Wash. Pub., No. 361, 125-162.

consisted of various types of white blood cells present in the peritoneal exudate of the guinea pig. These cells had been under observation in hanging drop preparations such as are commonly used in tissue culture work,[2] and since the usual methods of preservation seriously affected or entirely removed the vital stain, it was necessary to apply a new method. The one finally devised, as described below, not only retains the vital dye but permits other staining methods, such as Wright's method for differentiating blood cells, to be superimposed upon the vitally-stained cells.

In brief, the method consists in taking the cover glass from a hanging drop preparation in which the cells have been well stained vitally, either by direct contact with neutral red on the cover slip,[3] or by exposure to a dye bath consisting of a few crystals of neutral red dissolved in unbuffered Ringer's solution in the incubator at 38° to 40° C., and immersing the preparation for one half to one hour at 38° to 40° C. in the Ringer-formalin fixative, suggested by Fischer,[4] which is buffered as noted below. The tissue is then washed in running water for three hours, rinsed with distilled water and permitted to dry. After drying, the preparation may be counterstained with a one-tenth of one per cent aqueous solution of methyl green, as a nuclear stain, or with Wright's blood stain. The preparation is again dried and may then be mounted in damar, or, if necessary, it may be cleared according to Fischer's[4] method, by subjecting it, before mounting, to passage for two minutes each through a graded series of acetone-xylol mixtures consisting of 5 per cent, 30 per cent, 70 per cent, xylol, and two changes of pure xylol.

In order to obtain uniform results with this method and to insure the retention of the pseudopodial, granular, and vacuolar structures of the cells in the forms and relationships obtaining immediately before fixation, several precautions are necessary. First, all glassware used is chemically clean. Second, the granules within the cells are heavily stained with neutral red before fixation, otherwise, when counterstained with Wright's stain,

[2] Harrison, R. G., *J. Exp. Zool.*, 1911, ix, 787. Baitsell, G. A., and Sherwood, M. B., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 96.

[3] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277-88.

[4] Fischer, A., Tissue Culture, Studies in Experimental Morphology and General Physiology of Tissue Cells *in vitro*. Levin and Munksgaard, Copenhagen, 1925, 98-100.

they will appear bluish or black instead of red, making classification more difficult. Cells in which the granules are heavily stained from a deep scarlet through a cerise red even to an orange red, and in which the nuclei show no tinting with the dye, lend themselves most readily to permanent preservation and counterstaining by the present method. Third, in order to avoid the shrinkage or swelling or even the premature death of the cells, the variations in hydrogen ion concentration of the various reagents should not be great. It is our custom to use Ringer's solution from the same lot for the dye bath, for any necessary washing of the culture, and for the fixative. In all cases, the Ringer's solution is permitted to reach an equilibrium in hydrogen ion concentration by standing in the incubator at 38° to 40° C. for several days before use. In non-sterile Ringer's solution, whether made with distilled water or tap water, this equilibrium usually varies in pH value from 7.3 to 7.6. The formalin used is either acid formalin 37 per cent, or the same formalin kept over magnesium oxide. The buffers employed are either a phosphate-hydroxide mixture, pH 7.4; or primary and secondary M/15 phosphate mixtures, usually pH 7.4. The Ringer-formalin fixative consists of 20 cc. of Ringer's solution, 1.8 cc. of formalin and 2 cc. of buffer mixture. For general use, a final reaction from pH 7.3 to 7.6 is satisfactory, although, when simple fixation without the use of a dye bath is employed, a pH value as low as 6.8 gives as good results. The dilution of the Ringer's solution by the addition of formalin and buffer mixtures is negligible. Fourth, the use of the incubator during the dyeing and fixation processes insures a more rapid penetration of the cells by the reagents employed. Fifth, drying, instead of dehydrating by means of alcohol, avoids the loss of the alcohol-soluble neutral red and apparently has no ill effects upon the cells.

When Wright's method is employed to counterstain there is no confusion of the eosinophilic with the vitally stained granules; the former range from a bright pink to yellow, while the latter are red to reddish brown. Cells which did not show vitally stained granules before fixation do not present a granular appearance following the above treatment. Phagocytosis of red blood cells, eosinophilic and polynuclear leucocytes is beautifully demonstrated in the preserved preparations. On degeneration, there appears to be a tendency for the cytoplasm of the cells to assume acidophilic properties when subjected to Wright's stain, while

fragmentation of the nuclei is seen in those cells which were regarded as dead before fixation.

Further information as to the technical details, will be available later.

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Pseudobacteriophage of Bacillus anthracis.

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During the examination of some old stock cultures in November, 1924, there was found an agar slant of *Bacillus anthracis* which had the appearance of undergoing lysis by bacteriophage. The film of growth was interrupted by well defined partly confluent denuded areas, many of them containing small centrally located secondary colonies. Transplants of the culture to agar gave similar appearances after incubation for two days at 37° C. and one or more days at room temperature. Transplants to broth did not show clearing but the plaques again appeared when the broth cultures were transplanted to agar. An older agar slant culture from which the culture first mentioned above had been inoculated did not show plaques nor did fresh transplants of this culture, but when this "negative" strain was inoculated with material from one of the plaques of the "positive" strain plaques appeared in subsequent transplants. Filtrates of broth cultures of the positive strain were inactive when added to the negative strain.

Plaques on agar plates examined under the microscope were found to contain a nucleus of free spores, the remainder to the plaque being made up of pale remnants of bacilli and amorphous granular detritus. The matrix of growth surrounding the plaques was composed of typically curled but apparently spore-free anthrax filaments. Fishings from the centers of plaques yielded cultures of typical sporulating *Bacillus anthracis*. Fishings from the matrix yielded cultures of non-sporulating bacilli

otherwise like *B. anthracis,* hereafter referred to as the non-sporulating strain.

After prolonged growth on agar, on potato or in broth at 37° C. and at 20° C. the non-sporulating strain failed to produce visible spores and did not resist heating for twenty minutes at 75° C. Cultures of this sporulating strain resisted similar exposure to heat. Neither sporulating nor non-sporulating strain showed plaques when grown alone on agar. When the two were mixed plaques appeared. In the pure state the non-sporulating strain produced coarser curls than did the sporulating strain and in the mass the former growth had a somewhat more transparent appearance than the latter and was rather viscid to the touch of the needle. An agar slant inoculated over the entire surface with the non-sporulating strain and then touched in one or several places with the sporulating strain showed plaques where touched with the sporulating strain. Slants similarly inoculated with the sporulating strain, and then touched in spots with the non-sporulating strain, also showed plaques where touched with the latter. In the latter case, however, there were no spores in the centers of the plaques and central secondary colonies did not appear after further incubation.

When inoculated sub-cutaneously into mice both strains produced typical anthrax. For guinea pigs the sporulating strain was more pathogenic than the non-sporulating strain. Only the sporulating strain was pathogenic for rabbits. Passage of the non-sporulating strain through mice or guinea pigs and subsequent cultivation on agar or potato did not result in the appearance of spores. Neither did exposing bits of the infected spleens from animals in petri dishes at room temperature for several days induce spore formation.

When in mixtures of the two strains the sporulating strain was predominant, the non-sporulating colonies in young agar cultures presented the appearance of the well known so-called "pellucid spots" which have been described for a number of sporulating aerobes. Later these pellucid spots became sunken and had the appearance of plaques due to bacteriophage. On examining other strains of *B. anthracis* most of them were found to produce pellucid spots on agar. Another non-sporulating strain was isolated from one of these. In other cases there were obtained strains in which spore formation was greatly retarded

so that apparently the appearances described may be produced not only by mixtures of sporulating and non-sporulating strains but also by mixtures of rapidly and slowly sporulating strains.

The appearances described are similar to those described by Andrevont and Simon[1] and by Pesch[2]. A more complete description and discussion of our observations and those of the authors cited will appear in a subsequent publication.

3089

Chemical nature of some substances required for the growth of fibroblasts and epithelial cells

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Pure strains of fibroblasts or of epithelial cells increase in mass in an unlimited manner, when they are cultivated in plasma and embryonic tissue juice. For 14 years, colonies of a strain of fibroblasts have doubled in size every 48 hours in such a medium. Pavement and thyroid epithelium also have been found to manufacture unlimited amounts of protoplasm from the constituents of embryonic juice. Neither epithelial cells nor fibroblasts multiply in serum proteins, egg albumin, crystallized egg albumin, amino acids from embryonic juice, or artificial mxtures of amino acids for a longer time than in Tyrode solution. So far, embryonic juice is the only material which has been found to maintain epithelial cells and fibroblasts in a conditon of true cultivation.

Investigation of the chemical nature of the nutritive materials in the embryo juice has led to the conclusion that the nitrogenous substance utilized by the tissues is the protein itself. The amino acids and other ultra-filtrable and dialyzable constituents slightly stimulated the migration and multiplication of the cells, but failed to produce an increase in the mass of the tissues. Since the protein of the embryo juice is utilized by the cells, it seems evident

[1] Andrevont, H., and Simon, C. E., *Amer. J. Hyg.*, 1924, iv, 386.

[2] Pesch, K. L., *Centralbl. f. Bakt.*, Abt. I, Orig., xciii, 525.

that it must be hydrolyzed and some intermediate product absorbed. Complete digestion by trypsin and pepsin produced only toxic substances.

However, some of the higher cleavage products formed by the partial hydrolysis of protein have been found to produce the same effect as the protein of the embryo juice, causing continuous multiplication of cells and increase in the mass of tissue for long periods of time. In fact, some preparations of these protein hydrolytic products have given a larger increase in the mass of the tissue than has ever been obtained by embryo juice.

A most significant finding is that these growth-promoting proteolytic products have been obtained from foreign proteins as well as from embryo tissue. Egg white, commercial blood fibrin, and rabbit brain give excellent nutrient material on hydrolysis. In fact, the products of fibrin half digested by pepsin have given the best results thus far obtained. Witte's peptone is also capable of causing the multiplication of cells, resulting in an increase in the mass of tissue. Therefore, it is probable that no specific substance or hormone is required for the multiplication of cells, but only the proper nutrient materials.

Fractionation of these proteolytic products has been begun and has shown that the metaprotein has some action, but that proteose is more active. This proteose was obtained from Witte's peptone by first removing the protein, metaprotein, and any other products precipitated in 2.5 per cent trichloracetic acid, and then removing the peptones and smaller degradation products by precipitating the proteoses with sodium sulfate at 33° C., and reprecipitating four times and dialyzing. This purified proteose solution has caused continued growth of tissue *in vitro* and an increase in the mass of the tissue which is twice as great as that produced by embryonic juice. Tissues cultivated in digested fibrin have become four times as large as their control in embryo juice.

At last, the chemical nature of the nitrogenous substances which are used specifically by tissue cells in the process of multiplication has been discovered. The synthesis of protoplasm by these cells, when fed on proteoses, leads one to suppose that the marvelous effect of embryonic juice on tissue growth is merely due to a special condition of its proteins, which renders possible their splitting into proteoses by the action of the fibroblasts and epithelial cells.

3090

The influence on basal metabolism of some derivatives of di-iodotyrosine.

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The structure of thyroxin as recently determined by Harrington[1] represents the substance as a derivative of di-iodotyrosine and as containing two benzene rings. It is, therefore, of some interest to know what effect, if any, other derivatives of di-iodotyrosine containing two aromatic nuclei, would exert on basal metabolism.

Two such substances have been examined, namely, di-iodotyrosyl-di-iodotyrosine and the cyclic anhydride (diketopiperazine) of di-iodotyrosine. The former substance may be prepared by the action of iodine in feebly alkaline solution upon tyrosyl-tyrosine, or by the regulated action of alkali on the cyclic anhydride of di-iodotyrosine. The latter substances cannot be obtained by the direct action of iodine upon tyrosine anhydride, but may be readily attained by the action of iodine chloride in acetic acid upon the anhydride. Both substances are cream colored microcrystallin compounds, which in their solubilities and general chemical deportment, colour reactions, etc., show considerable resemblance to thyroxin. The anhydride of di-iodotyrosine melts at about 245 to 250 degrees (uncorr.) while di-iodotyrosyl-di-iodotyrosine does not melt sharply but begins to decompose above 200 degrees. The substances were prepared for these experiments by H. D. Dakin.

The metabolism experiments were carried out on a normal male individual of about 101 kilos, 184.5 cm. in height. All determinations were done in triplicate under basal conditions, either the Tissot or Benedict machine being used. All substances investigated were taken intravenously.

Basal metabolic rates determined on five days during the week preceding the injection of the cyclic anhydride of di-iodotyros-

[1] Harrington, C. R., Chemistry of Thyroxin. Communications: Biochemical Society of London, March 13, 1926.

ine varied between —9 and —12 per cent. Twenty-four hours after the injection of 21 mg. of the substance the basal rate was —9 per cent, and at the end of 48 hours was —7 per cent.

Three days following this determination the injection of 10 mg. of Squibb Thyroxin was followed at 24 hours by a rise to +4 per cent, which dropped to —1 per cent at 48 hours and remained for six days between 0 and —2 per cent.

On the seventh day after thyroxin 15 mg. of di-iodotyrosyl-di-iodotyrosine were taken, the basal rate staying at —2 per cent for the next 72 hours.

The di-iodotyrosyl-di-iodotyrosine used was prepared by the action of alkali on the cyclic anhydride of di-iodotyrosine, and the cyclic anhydride used by the action of iodine chloride in acetic acid upon the anhydride of tyrosine.

It is felt that the two substances investigated have shown no calorigenic activity.

3091

A study of the laxative action of wheat bran.

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The physiological action of wheat bran and a number of products isolated from bran was studied on dogs, which received as a basal ration a "synthetic" diet of casein, sucrose, and lard, together with the necessary mineral salts and vitamins. The purpose of the investigation was to determine what constituents of bran are responsible for its laxative effect. After a control period of 8 days the dogs were given the basal ration, supplemented with the bran or other material, the laxative power of which was to be determined. The frequency of defecation and the total weight of air-dried feces per eight-day period served as criteria of laxation.

Washed bran (starch-free) was laxative when ingested in amounts ranging from 10 per cent to 0.5 per cent by weight of the food intake. The average minimum effective dose was about

2 per cent (200 to 275 milligrams per kilo. body weight). About two grams of additional water was excreted in the feces for every gram of bran eaten.

The crude fiber of bran was a much more potent laxative than bran itself. The fiber when fed in such small daily doses as 0.5 gram to dogs weighing 7 to 9 kilos caused a marked rise in the number of defecations per eight-day period and produced an increase in the fecal output equal on the average to three times and, in some instances, to five or six times the weight of the ingested fiber. In general, bran itself did not possess this feces-forming power. Extensive experiments on 10 animals indicate that the laxative action of wheat bran is due at least in part to its crude fiber content.

The laxative factor of bran was not removed by acid and alkali hydrolysis or by prolonged, successive treatment with water, sodium chloride solution, hot 70 per cent alcohol, and weak sodium hydroxide solution.

A number of decidedly laxative products containing a relatively large proportion of crude fiber and pentosans were isolated from bran. The high crude fiber and pentosan content of these products may explain their pronounced laxative effect which was always as great as, and sometimes greater than that of the original bran.

Two dogs on a "10 per cent bran" diet utilized about 16 per cent of the nitrogen of the bran, but a third dog showed no evidence of the utilization of bran nitrogen. The addition of 10 per cent of bran to the control diet had practically no effect on the utilization of the fat and protein of the control ration.

Bran which had been washed with cold water until it was free of starch was a somewhat better laxative than whole (unwashed) bran.

Preliminary experiments indicate (1) that the crude fiber of bran is not utilized to any extent by the dog, and (2) that this crude fiber is a more powerful laxative than an equal amount of powdered agar.

3092

A procedure for the detection of allantoin in body fluids.

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None of the ordinary constituents of body fluids interferes with the test outlined below, save a substance in the urine, as yet unidentified, but which is probably a small amount of oxalic and perhaps glyoxalic acids, which have been reported as occuring freely in urine by Fürbinger[1] and by Granström[2], respectively. Creatinin, creatin, uric acid, etc. are negative. Allantoin, however, may be separated from the other constituents of the urine by precipitation with ammonical silver nitrate. Then the allantoin is subjected to the test. This procedure is necessary only in the case of urine for blood and other body-fluids show no interfering substance.

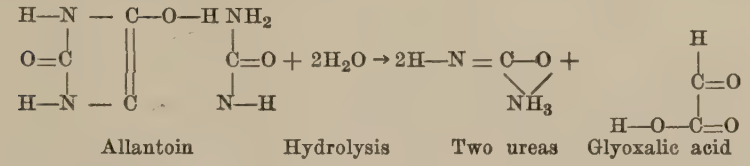
To 25 mg. filtered urine, add, if necessary, 10 per cent hydrochloric acid to slight acidity; if the urine is initially acid, this is unnecessary. Then add, drop by drop, 1 per cent aqueous solution silver nitrate until no more precipitate of silver urate is obtained; ten drops are usually sufficient. Let stand five minutes, filter, saving the filtrate. Or, one may use a 50 mg. centrifuge tube and precipitate by means of the centrifuge. A test for the presence of uric acid on this filtrate may be made by Folin's test. Then add, drop by drop, concentrated ammonium hydroxid to the filtrate to obtain the precipitate of allantoin silver nitrate; in case, after the addition of two drops of the ammonia, no precipitate appears, add a drop or two of silver nitrate solution as before, to insure that all of the silver nitrate first used was not carried down as silver urate. An excess of ammonia causes solution of the precipitate.

A precipitate, varying with the amounts of allantoin, may be obtained by centrifuging. Procedure for all fluids: To a few mls. of the fluid, or to the precipitate obtained above after decantation of the supernatant fluid, add one small flake of indol,

[1] Fürbinger, P., and Dunlop, J., *Deutsch. Arch. f. Klein. Med.*, 1876, xviii, 143-192. Dunlop, J., *J. Path. and Bact.*, 1895-6, iii, 389,429.

[2] Granström, E., *Hofmeister's Beitrage*, 1907-8, xi, 137-142.

a commercial product. To the fluid add, drop by drop carefully down the sides of the tube and inclining it, concentrated sulphuric acid, permitting the liquids to layer. If a precipitate of allantoin silver nitrate is used, dilute with about 2 mg. of distilled water before adding the sulphuric acid. At the point of junction of the acid and the supernatant fluid, a colored zone appears if the test is positive for allantoin, due to the hydrolysis of this substance in the warm acid to glyoxalic acid and urea and the condensing of the glyoxalic acid with indol in a manner similar to that in the Hopkins-Cole reaction for proteins, in which tryptophan affords the indol ring; the reaction is as follows (After Grimaux[3]):



If desirable, a 1:1000 per cent solution of indol in water may be used in place of the substance itself. Five drops of this solution may be used in the test. Like nearly all colorimetric procedures involving an aldehyde, this test does not lend itself to quantitative procedure.

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The lysis of dead bacteria by bacteriophage.

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There is no difference of opinion that only young and actively growing bacteria are subject to transmissible lysis. Old or dead bacteria do not undergo lysis and do not contribute to the increase in concentration of the active lytic substance, but rather, on the contrary, bring about a measurable reduction of it.

[3] Grimaux, E., *Ann. J. Chim.*, 1877, xi, 389.

Recently, Twort[1] has stated (somewhat similar to the evidence offered a year earlier by Gratia[2, 3]) that dead staphylococcus may undergo lysis if, in addition to a suitable bacteriophage, there is also present live staphylococcus. We have confirmed these observations, and in addition have endeavored to ascertain the explanation of the mechanism of this phenomenon. We found that in order to elicit the phenomenon it is necessary to control the numbers of live and dead bacteria in the mixture. An excess of dead bacteria interferes with lysis by adsorbing the active lytic substance before it has the opportunity to initiate lysis of the live bacteria, and thus all solution is prevented. The phenomenon is specific, that is, the lysis of live bacteria will initiate lysis of dead bacteria of the same species only. In its extent and completeness, lysis of dead bacteria occurs best with stapylococcus, the organism which easily undergoes autolysis under appropriate conditions. In the case of colon bacillus, which undergoes autolysis very slowly, the lysis of the dead bacteria is less regular and less complete.

If the experiment is performed so that a suitable semi-permeable membrane is interposed between the dead and live bacteria, dead bacteria are never dissolved, in spite of the lysis of living bacteria on the other side of the membrane. The active substance determining the lysis of dead bacteria is not diffusible, while the principle initiating the lysis of live bacteria diffuses freely and is demonstrably present on both sides of the membrane. Thus, transmissible lysis of bacteria can be shown to consist of two phases: The first, initiated in the live bacteria by the transmissible principle, which does not of itself cause lysis. In this phase this substance shows an increase in concentration. The second phase consists of lysis of bacteria with coincident setting free of another active principle. If dead bacteria are present in the immediate vicinity during this phase of the process, they too may be dissolved.

The following protocol is given in illustration of the above statements:

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[1] Twort, F. W., *Lancet*, 1925, ccix, 642.

[2] Gratia, A., and Rhodes, B., *Compt. Rend. Soc. Biol.*, 1923, lxxxix, 1171.

[3] Gratia, A., and Rhodes, B., *Compt. Rend. Soc. Biol.*, 1924, xc, 640.

Inside the membrane	Live staphylococci	Live staphylococci	Dead staphylococci	Dead and live staphylococci
Outside the membrane	Bacteriophage	Dead staphylococci Bacteriophage	Live staphylococci Bacteriophage	Bacteriophage
Results	Lysis inside	Lysis inside None outside	Lysis outside No lysis inside	Lysis inside of dead and live staphylococci

The ferment-like substance responsible for lysis of dead bacteria is easily adsorbed on the clay filter; it is heat labile, and is quickly inactivated on standing. In these respects it differs markedly from the substance to which transmissible lysis of live bacteria is due.

3094

Changes in viscosity during lysis of bacteria by bacteriophage.

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The fact has been observed that prior to lysis in the presence of the bacteriophage, bacteria usually undergo more or less marked swelling. The extent of swelling and the relative proportion of swollen bacteria, as well as the actual relation between swelling and lysis are difficult to establish by direct microscopic examination, because the swelling and lysis of bacteria go on simultaneously and continuously, and because the degree of swelling of individual bacteria varies to such an extent that results of such an analysis must, of necessity, be highly subjective. Moreover, in the case of cocci it is very difficult to decide whether or not swelling takes place at all.

It seemed, therefore, that since swelling of bacteria increases the relative volume occupied by the solids in the medium, the swelling of the total bacterial mass (with proper correction for growth) should bring about an alteration in viscosity of the solution.

Measurements were made both by means of a capillary viscosimeter of Ostwald and in the torsion viscosimeter of du Noüy. It was found that in general the viscosity of the mixture of bacteria with a corresponding bacteriophage increases steadily up to the time when visible lysis sets in, at which time the viscosity begins to diminish until it gradually reaches the original level.

In general, the greater the relative concentration of phage, the sooner the maximum viscosity is attained. The greater the number of bacteria (within the limits compatible with the concentration of phage present) the greater is the percentage of change in viscosity. At its maximum, the increase in viscosity of the mixture has varied in the experiments thus far performed, between 14 and 50 per cent, depending on the relative concentration of bacteria and bacteriophage. When calculated according to the formula of Kunitz[1], these results indicate an average increase of the volume occupied by bacteria of from 6 to 12 times. If, in place of living, susceptible bacteria, one employs a culture of homologous, resistant variant, or heterologous bacteria, or homologous, susceptible bacteria killed by heat, the viscosity remains unchanged. The heated bacteriophage which is devoid of its lytic power does not induce swelling of bacteria, and the viscosity of the original mixture remains unchanged.

3095

The production of antirachitic properties in human milk resulting from irradiation of the mother.

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Since it has been established that exposure to sunlight or to ultra-violet rays from artificial sources is able to protect animals or infants from rickets, the question arises whether this protective quality can be transmitted through the milk by the mother

[1] Kunitz, M., *J. Gen. Phys.*, May, 1926, Vol. ix, No. 6.

to the young. As the result of some experiments on cows, Luce[1] concluded that "the anti-rachitic value depends on the diet of the cow and possibly also on the degree of illumination to which she is exposed." More recently Steenbock, Hart and their associates[2] showed that the irradiation of a goat led to a decided increase in the antirachitic potency of their milk.

This is a question of importance in relation to the etiology of infantile rickets, for although this disorder occurs less frequently among nursing than among bottle-fed infants, nevertheless it has been found in from 1/3 to 1/2 of the breast-fed infants in the temperate zone. In the following experiments, undertaken to elucidate this question, rickets was primarily induced in a series of rats by means of the Sherman-Pappenheimer low phosphorus diet. After radiographs had shown that rickets was present, a ration of 25 cc. of human milk was substituted for the rickets-productive dietary. When the milk had been fed in this amount for a period of 9 days the animals were again radiographed, their blood analyzed for inorganic phosphorus, and the bones examined microscopically. It was thus found that this quota of milk failed to induce healing as evidenced by the radiologic as well as the microscopic picture. The inorganic phosphorus in the blood was very low—1.98 mg. per cent.

The woman was then irradiated every other day by means of a mercury-vapor lamp. The irradiation period at the outset was 4 minutes, and was gradually prolonged; the distance was 30 inches. After 5 treatments the distance was increased to 60 inches, and a constant exposure of 56 minutes established, which rendered the intensity approximately the same as during the initial period. After irradiation had been carried out in this manner for 1 month, the milk was fed to the rats daily in 25 cc. per capita amounts. As in the first part of the experiment, the animals had been previously rendered antirachitic by the low phosphorus ration.

A striking difference was noted between the results obtained from feeding milk which had been collected previous to or subsequent to irradiation of the mother. Whereas healing had not been brought about by means of the first milk, quite the reverse

[1] Luce, E. C., *Biochem. J.*, 1924, xviii, 716.

[2] Steenbock, H., Hart, E. B., Hoppert, C. A., and Black, A., *J. Biol. Chem.*, 1925, lxvi, 441.

was the case with the second milk, which in every instance produced marked calcification of the epiphyses. In several animals of the latter group the bones appeared almost normal. The inorganic phosphorus content of the pooled blood of these animals was 5.61 mg. per cent. It is evident that antirachitic properties had been transmitted to the milk in high degree as the result of the irradiation.

It seems that an experiment of this kind has a definite application to pediatrics. It clearly indicates the value of ultra-violet irradiation of the mother during lactation as a preventive of rickets in the baby. Probably during the winter months, when the infant is most in need of protection from rickets, the intensity of sunlight is insufficient to produce this property in the milk.

3096

Photopharmacology. V: Influence of sun's rays on growth of yeast in sodium benzoate.

DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

Growth of yeast cells in sugar solutions, in light and darkness was studied by measurements of CO_2 evolved. Uniform suspensions of Fleishman's baking yeast were made in solutions of glucose or more often of sucrose, and the growth in sunlight and in the dark at the same temperatures was studied in two sets of fermentation tubes. Ordinarily suspensions of 0.5 or 1 per cent by weight of yeast in a 5 per cent or 10 per cent solution of sugar was employed. It was found that fermentation took place more rapidly in the dark than in the light, at the same temperature. The effect of sodium benzoate on the growth of yeast and fermentation was studied by adding the drug in concentrations of 1:1500 to 1:500. It was found that sodium benzoate in concentration of 1:1000 produced practically no inhibition in the growth as compared with normal yeast suspensions when kept in dark, and that even concentration of 1:500 of ben-

zoate caused but very slight inhibition of growth in the dark. On the other hand when exposed to direct sunlight even 1:1000 and 1:1250 of sodium benzoate produced a distinct retardation of fermentation, and the degree of inhibition was very much greater than in control experiments with sunlight alone, without the benzoate. The inhibitory action of the sodium benzoate was increased a hundred and more per cent in the light. In diffuse sunlight, growth of yeast in benzoate was also less than in darkness at the same temperature.

Examination of the glass from the fermentation tubes by spectro-photography with a mercury vapor quartz lamp revealed that it was permeable to wave lengths as short as 3000 Angstrom units. By the use of suitable filters in connection with the above experiments it was found that it was the shorter rays of the sun, that is, those which were cut out by amber and brown colored filters which were responsible for the above photosensitizing effect on sodium benzoate. The effect of adding certain fluorescent dyes to benzoate solutions was also studied. Addition of esculin 1:100,000 *decreased* the inhibitory action of sodium benzoate. On the other hand, solutions of eosin, *markedly potentiated* the inhibitory action of sodium benzoate on yeast fermentation. The above experiments were carried out in the bright sunlight on sea shore at Ocean City, Md., during the summer of 1925, and additional experiments with quartz lamp and spectroscope were performed in the laboratory.

3097

Photopharmacology. VI: Influence of sun's rays on growth of yeast in some fluorescent solutions.

DAVID I. MACHT.

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The influence of a number of fluorescein derivatives on the growth of yeast in sunlight and in the dark was studied, in a manner similar to that described in the preceding communication. A 0.5 per cent or 1 per cent suspension of yeast in 5 per cent or 10

per cent of cane sugar was generally employed and the quantity of CO_2 evolved was measured. To such suspensions of yeast in sugar solutions, the following fluorescent compounds were added in concentrations varying from 1:50,000 to 1:250,000.

The sodium salt of fluorescein itself; the potassium salt of tetra-brom-fluorescein (eosin); the potassium salt of di-brom-fluorescein; the sodium salt of tetra-iodo-fluorescein (erythrosin); the potassium salt of tetra-chlor-fluorescein, the chlorine being introduced into the phthalic residue; the potassium salt of a chlorinated fluorescein with the chlorine introduced into the resorcin component; and the potassium salt of sulphone fluorescein. These compounds were prepared at the request of the author by Dr. E. White through the courtesy of the research laboratories of Hynson, Westcott, and Dunning Co.

It was found that in concentrations 1:100,000, none of the dyes produced any appreciable effect in yeast fermentation in the *dark*. In stronger concentrations (1:50,000) eosin produced a slight inhibition. When exposed to direct sunlight, in concentrations of 1:50,000 all of the dyes produced a slight inhibition of the yeast. When however, the same dyes were added to yeast suspensions in direct sunlight together with small quantities of sodium benzoate a remarkable synergistic effect was noted. The inhibitory power of the combinations on the fermentation power of the yeast was potentiated two hundred and even more per cent, as compared with either dye or sodium benzoate alone. Thus solutions of eosin 1:100,000 plus sodium benzoate 1:1000, in direct sunlight produced an inhibition two or three times as great as that produced by either eosin or sodium benzoate alone. Such a potentiation was not noted in the dark.

On comparing the relative potency of the various fluorescein derivatives in combination with sodium benzoate, it was found that eosin or tetra-brom-fluorescein was more effective than tetra-chlor-fluorescein, and the latter more powerful erythrosin. The tetra-brom compound was more powerful than the di-brom-fluorescein. The chlorinated compound with the chlorine in the phthalic residue was more effective than the compound with the chlorine in the resorcin group, and the sodium fluorescein and sulphone fluorescein, were the weakest of all. On comparing the tetra brom with the tetrachlor fluorescein, it was found that whereas in direct sunlight the eosin was more effective, this dye after a few hours was for the most part decolorized, so that after ex-

posure for longer than 4 hours, it became relatively weaker than the chlorinated compound.

By the use of colored filters it was found that the shorter rays of sunlight were the more potent in producing the above photochemical potentiation. The glass of the fermentation tubes was tested with a mercury vapor quartz lamp and spectroscope and was found to transmit waves down to about 3000 Angstrom units. Most of the experiments with sun's rays were performed in the open, on the coast of the Atlantic Ocean at Ocean City, Md., during the summer of 1925.

3098

Experiments with trypanosomes in relation to the Wassermann reaction.

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The results of recent work[1] on the possibility of producing anti-bodies by means of substances apparently belonging to the class of lipoids have stimulated renewed investigation on the cause of the production of the Wassermann reagins.

Several main hypotheses relative to this subject have been considered. One of these implies that the reagins are no antibodies at all; others suppose that they are antibodies for spirochetes with an affinity also to lipoids of common origin; still another assumes that antibody formation is brought about by lipoids of the infected organism.

In analogy to the experiments on the production of heterogenetic antibodies by mixtures of proteins and alcoholic extract of organs,[1] Sachs and his coworkers[2] thought of the possibility that the production of antibodies is due to a combined action

[1] Landsteiner, K., and Simms, S., *J. Exp. Med.*, 1923, xxxviii, 127; Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1925, lxi, 427; Landsteiner, K., *Biochem. Z.*, 1921, cxix, 306.

[2] *D. med. Woch.*, 1925, No. 15.

of components of the spirochetes with lipoids derived from the infected animals. As a support of this hypothesis they report experiments in which it is shown that Wassermann positive sera can be produced in rabbits by the injection of alcoholic extracts of rabbit organs along with diluted pig serum.

Among our own experiments dealing with the question were such concerning the possibility of obtaining Wassermann positive sera by injections of dead trypanosomes *(T. equiperdum)*. It had been shown previously that infection of rabbits with trypanosomes *(T. equiperdum)* often results in the development of a positive Wassermann reaction.[3] Experiments by Klopstock in which he succeeded in eliciting Wassermann reactions by injecting dead *Spirocheta pallida* have been published very recently.[4]

For the selection of rabbits the Sachs-Georgi flocculation test was employed and only such whose sera gave completely negative reactions were taken for the experiments. One set of animals was treated with dead trypanosomes which had been kept over night in the icebox in a saline solution containing 0.25 per cent phenol. The quantity injected each time approximated 6 to 7 milligrams dry weight. Another set of rabbits was infected with a small dose of trypanosomes resulting in a slowly developing disease.

Leaving further details for a subsequent publication, a brief summary of the results is given in the following table. The titration of the sera in the complement fixation tests were made by halves. (0 indicates no hemolysis.)

The table records the results one month after the infection and after 4 to 7 injections of dead trypanosomes. In most cases strong reactions occurred after 3 to 4 injections.

The facts reported show that dead trypanosomes are powerful agents for inducing the formation of Wassermann reagins. They were active in distinctly smaller quantities than those of tissues or lipoids of animals used in the experiments quoted.

From the experiments of Klopstock with Spirochetes and ours with trypanosomes it would seem superfluous to assume that lipoids of the body along with components of the microbes are the operative agents in the production of the Wassermann reagins since the microbes themselves suffice to produce the effect.

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[3] Landsteiner, K., Müller, R., and Pötzl, O., *Wien. klin. Woch.*, 1907, No. 46.

[4] Klopstock, F., *Deutsch. med. Woch.*, 1926, p. 226.

TABLE I.

No. of animals	Wassermann reaction with cholesterinized beef heart extract.	Sachs-Georgi flocculation.	Complement fixation with lecithin	Flocculation of lecithin	Complement fixation with alcoholic extract of Trypanosomes
		Rabbits Injected with dead Trypanosomes.			
831	0, 0, tr, v str.	+++	ac, c, c.	0	0, 0, str, c.
834	0, 0, 0, d, ac, c.	+++	c, c, c, c.	0	0, 0, 0, str, ac.
836	0, 0, 0, 0, str, c.	++++	str, v str, ac, c.	0	0, 0, 0, 0, str.
837	w, d, str, c.*	+*	v str, d, str, ac, c.	f. tr.	0, 0, w, ac, c.
838	0, 0, 0, 0, d, c.	+++	ac, ac, c.	0	0, 0, 0, tr, c.
839	0, 0, 0, 0, 0, str.	+++	w, 0, w, ac, ac, c.	+	0, 0, 0, 0, v str.
840	0, 0, 0, v str, c.	+++	d, str, c.	±	0, 0, 0, str, c.
841	d, w, str, ac, c.	++	c, c, c.	0	0, 0, f, tr, ac, c.
		Rabbits infected with Trypanosomes.			
821	0, 0, 0, 0, str, c.	+++	0, 0, 0, str, c.	++±	
822	ac, ac, ac, c	+±	c, c, c.	±	
823	str, str, ac, c.	+	0, 0, c.	+±	
824	0, 0, 0, ac, c.	+++	0, 0, f.tr, ac, c.	++±	
825	str, str, v str, c.	+±	w, 0, w, ac, ac, c.	++	
826	0, 0, 0, tr, c.	++±	0, 0, 0, 0, ac, c.	++	
827	0, 0, 0, c.	+	0, 0, v str, c.	+±	
		Rabbits infected with Syphilis.			
5726	0, 0, 0, d, c.	+++	0, 0, d, ac, c.	++±	
5570	tr, str, ac, ac, c.	+++	ac, c.	0	
5732	0, 0, w, c.	++±	0, tr, str, ac, c.	++	
5575	0, w, v str, c.	f. tr.	0, v str, c.	trace	
		Normal Rabbits.			
1	c, c, c, c.	c, c, c.	0	0	c, c, c, c.
2	c, c, c, c.	ac, c, c.	0	0	c, c, c, c.

* Tests after 7 injections. The reactions with this serum were strongly positive after 4 injections.

Whether or not the changes in the serum caused by infection are in all respects identical with those following the injection of the killed microorganism still remains to be investigated more fully. In our experiments there was a difference in the reaction when the sera from infected rabbits and from those treated with dead trypanosomes were tested with organ extracts and egg lecithin respectively as is seen from the table. Also the sera of the infected animals exhibited a strong agglutination of the live trypanosomes. In order to determine whether these differ-

ences are constant, repeated examinations at several stages during the course of the experiments seem necessary and also the investigation of infections of varying degrees of severity.

We are indebted to Dr. Kolmer for supplying us with a strain of trypanosomes, and to Dr. Brown and Dr. Pearce for the sera of rabbits infected with syphilis.

3099

Relation of spinal level of blood pressure to successive occlusions of head arteries in cats.

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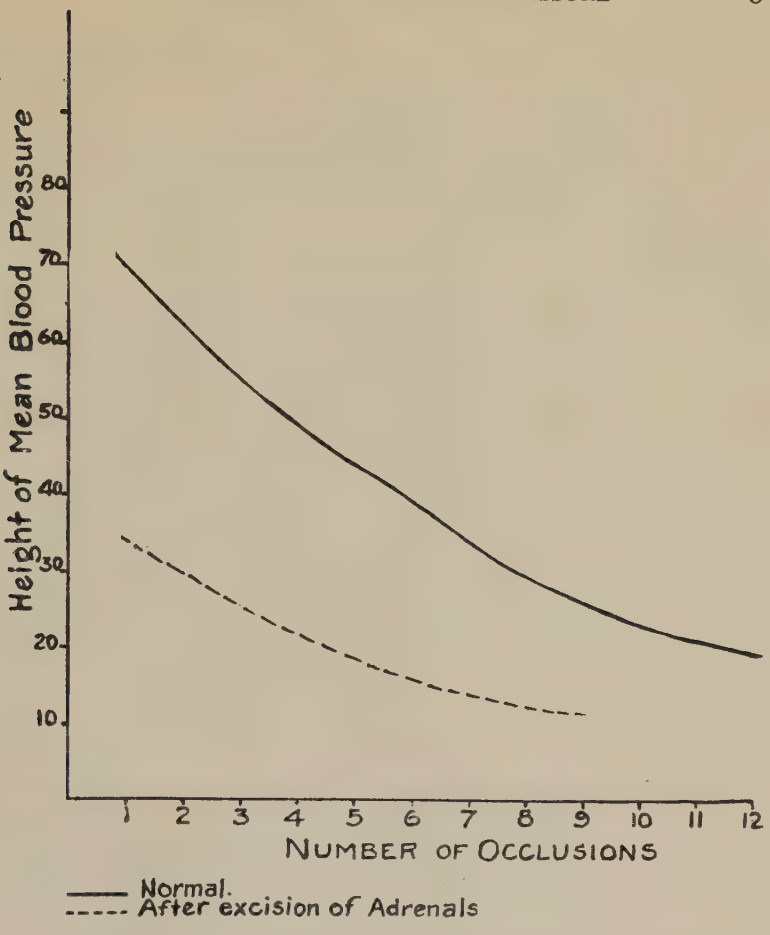
In experiments upon the cardio-vascular responses following repeated occlusions and restorations of the circulation of the head arteries in cats, it has been repeatedly observed that the spinal level of blood-pressure, that is, the level of pressure maintained by the activity of the spinal cord alone, when the functional activity of the medulla has been eliminated, may vary between 35 and 75 millimeters of mercury.

Certain criteria are necessary to determine whether blood-pressure within this range is actually spinal pressure, since in some animals, a pressure as low as 50 or 60 mm. is sufficient to restore medullary activity, whereas in other animals, a pressure to 70 to 75 mm. may be ineffectual in bringing this about. Blood-pressure, then, may be considered spinal when

(1) its level is unaffected by occlusion or release of the head arteries.

(2) no signs of any change in level of pressure or return of any medullary reflex is observable from half to three quarters of an hour after circulation has been restored to the head arteries.

With these conditions in mind, a series of experiments was carried out, with varying numbers of successive occlusions, from 1 to 14, in normal cats. In these animals, at some stage in the occlusion series, the head arteries were permanently ligated fol-



lowing an anaemic rise and fall of blood pressure. Blood pressure recorded on the chart in each case, is the mean pressure.

In these experiments, it was found that the height of the spinal pressure has a definite relationship to the number of occlusions which have been done on the cat, as the accompanying curve shows. That is, when the first or second occlusion is permanent, spinal pressure is about 70, whereas, when the 13th or 14th occlusion is permanent, the spinal pressure is about 20 mm. These observations have been repeated until one can predict what the spinal pressure after any given occlusion will be.

In cats in which the adrenals were excised prior to the occlusions of the head arteries, a similar relationship between height of spinal pressure and number of occlusions, is observed. But

a lower level of pressure occurs after any given occlusion. This also, is indicated in the accompanying chart.

During these experiments, after spinal pressure had been for some time maintained, curare was given intravenously. By this means, whatever rôle the skeletal musculature plays in the maintenance of spinal blood pressure, was eliminated. Pike[1] and Langley[2] have shown that the intravenous injection of curare is followed by a fall of the spinal blood pressure, indicating some participation of the skeletal muscles in the maintenance of this pressure. Pike has further shown that the fall of pressure induced by curare is about equivalent to that seen after anatomical division of the dorsal roots of the spinal nerves in the cat. In these experiments, the injection of curare was followed by a fall of blood pressure, which in each individual case was about 40 to 45 per cent of the mean spinal pressure. This would indicate that the progressively lower pressures with increasing numbers of occlusions in the same animal are due to a progressive failure of function of the somatic neuro-musculature as well as of the vascular neuro-muscular mechanism.

3100

The effects of repeated intravenous injections of distilled water on the blood picture in rabbits.

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The effect of repeated induced intravascular hemolysis upon the blood of rabbits was studied in the following manner: Ten to 12 cc. of sterile distilled water were injected slowly into the marginal ear veins of 6 rabbits at 48 hour intervals. The number of injections varied, the maximum being 34. Recently, as a control experiment, Patterson and Kast[1] described an anemia in

[1] Pike, Suart, *J. Exp. Physiol.*, 1913, vii, 1.

[2] Langley, *J. Physiol.*, 1919, liii, 120.

[1] Patterson, M. D., and Kast, L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 172.

rabbits produced by the intravenous injection of sterile distilled water, but as their experiments were only continued for 19 days, they apparently did not observe that the animals subsequently developed an increased resistance to such injections.

The following constituents of the blood were studied; erythrocytes, hemoglobin, leucocytes and reticulocytes. The resistance of the erythrocytes against hypotonic salt solution was also determined. The examinations were made immediately preceding each injection. Control observations were made upon normal rabbits and rabbits which had received physiological salt solution in doses corresponding to the distilled water.

After various intervals following the repeated injections of water, there was a diminution of 40 per cent to 50 per cent in the number of erythrocytes. However, despite continued repeated injections the erythrocyte count returned approximately to the original figures. There was a slight to moderate polychromatophilia and anisocytosis with an occasional normoblast and hemacytoblast. The number of reticulocytes showed a slight increase; the highest figure was 7 per cent. The variations in the percentage of hemoglobin were less marked than in the number of erythrocytes. The total number and differential count of the leucocytes remained within normal limits. The resistance of the erythrocytes against hypotonic salt solution did not change beyond the limits of normal variations in rabbits.

After having recovered from the anemia despite continued injections of distilled water in 10 to 12 cc. doses, an attempt was made to determine the tolerance of these rabbits for increased amounts of water. They varied markedly in their reactions. One rabbit died after 30 injections of 12 cc. each, without any increase in the dose. This animal died 24 hours after the last injection and showed a progressive paralysis of the extremities. Another rabbit which had received 33 injections of 12 cc. of distilled water died in a generalized convulsion immediately after a second injection of 50 cc. On the other hand, in a third rabbit which had received 32 injections of 12 cc. each, the dose was increased until 250 cc. were injected at one time without any demonstrable reaction. This animal received a total of 1900 cc. of distilled water during a period of 35 days before it succumbed after an attempt to inject 300 cc. Death was due to pulmonary edema.

Untreated normal rabbits varied in their response to injections of distilled water. One rabbit died immediately following a single injection of 20 cc. while another withstood the injection of 250 cc. on two consecutive days without a reaction but died on the third day immediately after an attempt to introduce 300 cc. into the circulation. Hemoglobinuria occurred in all the rabbits which received large amounts of distilled water.

There were no striking changes found in the organs except those incidental to increased blood destruction. Large numbers of pigment laden phagocytes were found in the sinuses of the spleen and lymph nodes. When very large doses of distilled water were injected, the sinuses of the spleen and liver were distended with the shadows of erythrocytes; there was also evidence of pulmonary edema. The bone marrow showed no significant changes.

3101

Some limitations of the action of the so-called follicular hormone in birds.

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During the past twenty-five years the work of many investigators has shown that the ovaries exercise control over the rapid temporary growth and hyperemia of the uterus which is characteristic of "heat" in mammals. Various extracts made from ovaries, and from other distinct tissues, have also long been known to bring about these particular uterine changes when injected into mammals (Marshall and Jolly, 1905; Lane-Claypon and Starling, 1906; Sonnenberg, 1907; Adler, 1911). The separation from the ovary of a lipoid fraction capable of inducing these uterine changes is also well established (Iscovesco, 1912; Hermann, 1913; Fellner, 1913, 1921; Seitz, Wintz and Fingerhut, 1914; Frank and Rosenbloom, 1915); and recently both the separation and the testing of this hormone have been

more satisfactorily accomplished (Allen and Doisy, 1923; Frank and Gustavson, 1925). Still other hormones have probably been separated from the ovary (Iscovesco, 1912; Seitz, Wintz and Fingerhut, 1914; Papanicolaou, 1924), but the one considered here is the utero-stimulant" of Iscovesco, "das spezifische Ovarialsekret" of Fellner, the "follicular hormone" of Allen and Doisy, and the "female sex hormone" of Frank and Gustavson.

No previous study has been made of the action of this hormone when injected into a bird—nor into any animal other than a mammal. Allen, and co-workers[1], however, obtained from fowl ovaries an extract which gave positive tests for the hormone when injected into rats. They were unable to obtain the hormone from egg-yolks. Fellner[2] extracted the hormone from the egg-yolks of the fowl and from the ovaries of fish, and obtained the typical uterine reactions in tests made on rabbits. In our rather limited studies we have obtained positive results on oviducal enlargement (little or moderate) and hyperemia in 3 of 7 tests with immature (3.5 to 6.5 mo.) ring doves. Oviducal weights from 13 controls for the three positive tests indicated an enlargement of the latter by from 10 to 60 per cent; the hyperemia was unquestionable. The lack of "specificity," as between birds and mammals, of this particular action of the hormone or substance is therefore now completely established.

Of equal or greater interest in our results are the apparent limitations of the action of this hormone in birds. Four of the seven immature doves (weight, about 150 gm. each) tested were quite unaffected although the dosage used probably ranged from 5 to 25 rat units. Three of the four doves which showed no response were given somewhat larger absolute amounts than that simultaneously given to two virgin rabbits aged 4 months. The weight of a rabbit was about ten times that of a dove, but the response was obtained in both rabbits and in neither of the doves. In two of the three positive results with doves a dosage of 5 rat units of purified hormone from the placenta* was given during 3 days and autopsies made one day later; in the third, a less marked response was obtained from approximately 22 rat

[1] Allen, E., Whitsett, J. W., Hardy, J. W., and Kneibert, E. L., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 500.

[2] Fellner, O. O., *Klin. Wochenschr.* (Berl.), 1925, iv, 1651.

* Supplied through the courtesy of Drs. R. T. Frank and R. G. Gustavson.

units† from liquor folliculi of sow administered during 6 days. This limited response under high dosage, together with the complete failure of relatively very heavy dosage to induce oviducal changes in some of our birds with the same preparation which (in relatively and absolutely smaller amounts) gave positive results in rabbits, probably indicates a less effective action of the hormone on the oviducts of birds than in those of mammals. This limitation, however, may be correlated with the fact that the functional ovaries of birds are relatively much larger than those of mammals. In other words, the ovary of the bird may normally supply the organism with larger quantities of the hormone than does the ovary of the mammal.

Still other limitations of the action of this hormone appear if this same hormone is considered as being capable also of inducing "puberty," of immediately reinforcing or increasing the growth of the larger follicles in the ovary, and of generating female sex behavior. From our knowledge of the rate and time of development of oöcytes in the ovary of the dove or pigeon, we know that injections and observations in these forms would have to include a period of nearly four weeks before an induced ovulation could be expected in an immature dove aged two or three months. We believe that ovulation is the only adequate criterion of puberty, and that conclusive proof of the capacity of this hormone to induce premature puberty in either birds or mammals is the most important theoretical point remaining for solution in work with this substance; but unfortunately our limited supply of follicular fluid has thus far prevented any adequate test of this matter. Bearing on this point nevertheless is the impression we get of rather wide-spread *degeneration* of the larger follicles in the ovaries of most (not all) treated birds.

The following facts also bear on the topic last-mentioned above. The capacity of the hormone to cause a continued growth and ovulation of ova in the ovary of mature birds has been insufficiently examined, but with entirely negative results. Four such ovulating females were tested with relatively fresh (and mostly otherwise tested) preparations. The first was given 22 rat units in 15 injections during a period of 6 days. The second was given the crude alcoholic extract from about 28 cc. of folli-

† The purified hormone for this injection was kindly supplied and tested by Dr. Edgar Allen.

cular fluid in 20 injections during 7 days. The third was given the purified hormone from about 16 cc. fluid in 14 injections during 5 days. The fourth was injected during the first 7 days of her incubation period. In none of these four cases was there any suggestion of an increase in the rate of maturation and release of ova. Possibly an action of this sort is not to be expected in the case of the pigeon, but it is certainly a fact that something connected with *season* does affect changes in ovulation rate in these animals. Two additional tests were made on older females, one a virgin of advanced age, and the other a bird that had stopped laying 3.5 mo. before beginning injections; results were negative in both cases.

The hormone or substance used by us (except as noted in footnote 3) was obtained from the liquor folliculi of the sow. It was kindly supplied by Dr. F. Fenger, of Armour and Co., Chicago, and reached us on the third or fourth day after collection and storage in two volumes of alcohol. A part of this alcohol filtrate was evaporated within a few hours at low temperature (less than 50° C.) and the injection of this fraction begun at once. The remainder was purified by the Allen-Doisy method within three days and injections begun immediately in order to avoid well known rapid loss of effectiveness of this substance. After preparation the hormone was dissolved in mazola (one lot in glycerin) and kept *in vacuo*. The material was injected subcutaneously (intraperitoneal in 1 series). Young male doves were also treated, but the results of that study, together with additional data for the female tests, will be described elsewhere.

SUMMARY

Brief reference is made to the more important earlier studies on the action of a utero-stimulating substance in mammals.

In a small group of tests of the utero-stimulating action of a placental extract, and of a substance prepared from the liquor folliculi of the sow by the Allen-Doisy method, we have obtained a few positive results—enlargement and hyperemia of the oviduct of virgin doves. This supplies the last necessary fact in proof of the lack of specificity, as between birds and mammals, of this substance.

Heavy dosage failed to affect a response in the virgin oviduct in some cases. Responses in the ovary and on sex behavior in virgin and mature doves have not been observed. An insufficient

number of cases was studied but our results indicate a less ready, and a less varied, response of the bird to this substance than has been found for mammals.

3102

Heavy alcoholization and prenatal mortality in mice.

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A previous investigation[1] has shown that treating female mice with light doses of alcohol fumes (beginning at 4 weeks, every day for 45 minutes in a pint bottle with 3 cc. alcohol) does not modify the prenatal mortality or any other phase of their reproduction. In the present study the same technique for administering the alcohol has been used, but each mouse was left in its bottle until in a state of deep anesthesia 5 days per week. This required 1 to 2 hours for mature mice. Experimental animals were mated at 4 weeks, and on the day of birth the mothers were returned from their isolation in pregnancy boxes to mating pens, without their young. The results are based on the number of corpora lutea associated with each successive litter of young born; the difference between the number of corpora lutea and the number of young is called the prenatal mortality. The technique of counting the corpora lutea has been described[2] and the reliability of this criterion of prenatal mortality discussed[3].

Two main series of experiments have been performed. In the first series the mothers only were treated (data from 657 litters). The unit experiment was a litter of 4 to 6 females half of which were treated. All lived in the same pen, mated to the same male, an older sib previously found fertile.

In the second series of experiments the fathers only were

[1] MacDowell, E. C., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 480.
[2] MacDowell, E. C., *Anat. Rec.*, 1924, xxvii, 329.
[3] MacDowell, E. C., and Lord, E. M., *Am. J. Anat.*, 1926, xxxvii, 127.

treated. The unit experiment[4] consisted of 2 treated and 2 control males from the same litter and 16 to 20 females from a different stock 2 to 3 weeks older than the males. They were divided equally between the 4 males for their first litter, and mated in turn with each of the other males for successive litters, always alternating treated and control males. In four of the unit experiments males from the inbred Bagg albino line were used (data from 452 litters); in four other experiments males from the still more inbred Dilute brown line were used (data from 387 litters).

	Order of litter.							
	1st	2nd	3rd	4th	5th	6th	7th	8th
Females treated								
Treated litters	64	63	57	46	33	22	10	7
Per cent P. mort.	45.2	51.7	55.7	61.8	69.7	74.4	74.3	67.9
Control litters	67	66	61	54	46	37	15	9
Per cent P. mort.	32.6	43.1	50.2	50.3	58.1	63.3	74.7	64.4
Males treated								
B. alb. treated litters	50	46	47	33	24	9	9	3
Per cent P. mort.	26.3	25.5	38.3	39.5	55.3	69.5	68.6	84.6
B. alb. control litters	57	53	38	34	16	19	6	8
Per cent P. mort.	22.2	28.6	40.9	39.3	63.1	58.7	64.8	75.7
D. br. treated litters	47	39	41	27	17	7	7	3
Per cent P. mort.	27.9	46.2	46.0	54.3	69.4	49.2	71.6	55.6
D. br. control litters	49	48	32	35	11	13	4	7
Per cent P. mort.	33.7	34.8	43.6	51.5	46.5	67.2	55.6	72.7

Since the order of the litter has been found[5] to have a strong influence on the amount of prenatal mortality, the summary given is subdivided according to parity. The figures in the body of the table are the number of litters and the per cent prenatal morality calculated from the total number of corpora lutea and young.

The prenatal mortality of the treated mothers is greater than for the controls in every case but for 7th litters; 1st, 4th, 5th and 6th litters give differences greater than 10 per cent. Treatment of the females with heavy doses of alcohol increases the prenatal mortality of the embryos.

Treatment of the Bagg albino males appears to have no con-

[4] MacDowell, E. C., Lord, E. M., and MacDowell, C. G., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 517.

[5] MacDowell, E. C., and Lord, E. M., *Anat. Rec.*, 1924, xxix, 141.

sistent effect on the prenatal mortality. With the exception of the per cents for 6th, 7th and 8th litters, which have the smallest numbers, the prenatal mortality for litters from the test and control males is very nearly the same.

The prenatal mortality for treated Dilute brown males is higher than the controls for the 2nd, 3rd, 4th, 5th and 7th litters, but counting only equal numbers of litters from each female from treated and control fathers, and ignoring parity, the per cent prenatal mortality for litters from treated fathers is 0.1 per cent less than the controls. A similar summary for Bagg albino males shows the treated fathers give litters with 1.01 $\pm$ 0.82 per cent more prenatal mortality.

It is concluded that no clear effect of the heavy treatment of the fathers can be found on the prenatal mortality in their litters. On the other hand, a difference in prenatal mortality is found between the litters from Bagg albino and Dilute brown males whether they are from treated or control fathers. Since all the females were from the same, third, strain and the methods of the experiment seem effectively to remove any other explanation, the greater prenatal mortality of the Dilute brown males may be accepted as due to a strain difference manifest in the sperm.

No other work is known in which prenatal mortality as here defined has been used as the criterion of the effect of any treatment, although various authors[6] have reported prenatal mortality based on autopsy counts of corpora lutea of pregnancy in various animals. Stockard[7] gives data on prenatal mortality in alcoholic guinea pigs but this is based only on deaths of foetuses large enough to be counted by palpation. He reports that the treatment of the fathers alone increases the prenatal mortality as much as the treatment of the mothers alone. It is clear that this conclusion is not supported by the present results from mice.

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[6] Hammond, J., *J. Agric. Sci.*, 1921, xi, 337; Corner, G. W., *Am. J. Anat.*, 1923, xxxi, 523; Long, J. H., and Evans, H. M., *Mem. Univ. Cal.*, 1922, vi; Parkes, A. S., *Proc. Roy. Soc.*, 1923, xcv, 551.

[7] Stockard, C. R., *Proc. Am. Phil. Soc.*, 1923, lxii, 311.

3103

Toxicity of filtrates of B. Friedlander.

EMERSON MEGRAIL.

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A number of investigators have made studies on filtrable toxic products produced by bacteria in young cultures. These products have been variously called soluble toxins, X substances and endotoxins. In order to produce constant effects it has been necessary to inject the filtrates intravenously into rabbits and mice. The use of a small laboratory animal and a method of inoculation which would allow a somewhat greater range in the materials introduced would aid in a more detailed study concerning the nature of these substances.

It was thought that an organism more highly virulent for the mouse than those used by other observers might produce a filtrate in young cultures which would be toxic intraperitoneally. A strain of *B. Friedlander* (non-lactose-fermenting) was chosen which consistently caused death in about 12 hours when introduced intraperitoneally into mice. This culture was grown for various periods in veal infusion Witte peptone broth with and without 0.1 per cent dextrose. Sterile filtrates of these cultures were used for injections.

Eighteen-hour cultures produced no effect on the mice. Older cultures, 42 hours, 66 hours, 5 days, 10 days, and 20 days, while more toxic, did not produce constant effects. In some cases mice receiving 1 cc. of the filtrate survived, while others receiving 0.5 cc. died in 8 to 24 hours.

Eighteen-hour cultures grown anaerobically, and aerobic cultures in Martin's media, produced no effect in the animals. The intraperitoneal injection of such substances as gum tragacanth, bile, and 1:500 calcium chloride, as peritoneal irritants, followed by the injection of a 42-hour filtrate likewise caused no reaction.

Rabbits inoculated intravenously with 3 cc. of the filtrate of this strain, grown in veal infusion Witte peptone broth, did not show any signs of discomfort with the 18-hour cultures. One rabbit of three showed marked diarrhea in one hour after injection from the 42-hour culture but no prostration or dyspnea such

as have been demonstrated with the colon-paratyphoid group and other organisms. Rabbits inoculated with 3-day filtrates were not affected. One rabbit inoculated with an 8-day filtrate had a severe diarrhea within one hour after injection. A guinea pig received intravenously 1 cc. of the filtrate of the same strain which affected the rabbit, but showed no signs of discomfort. Rabbits injected with filtrates of another strain of *B. Friedlander* which was not virulent for mice were not affected.

The results indicate that with an organism highly virulent for mice when injected into the peritoneum, injections of filtrates of culture 18 hours to 20 days old could not be shown to be toxic for these animals when injected into the same body cavity. The effect of the filtrate of *B. Friedlander* appears to be less constant in its action in rabbits when given intravenously than that reported with other organisms.

3104

Heparin. II. Investigation of possible antigenic action.*

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In a previous paper[1] were reported details of experiments on the intravascular use of heparin[2, 3, 4] in etherized dogs. These experiments, together with other experiences in preservation of blood samples used for various physical and chemical analyses, have demonstrated the value of this material in laboratory work. It seemed advisable, therefore, to investigate the possibilities of its use in transfusion when, for any reason, there might be delayed injection.

* Part of the expense of this investigation was borne by a grant from the American Association for the Advancement of Science.

1 Reed, C. I., *Am. J. Physiol.*, 1925, lxxiv, 79.

2 Howell, W. H., *Am. J. Physiol.*, 1918, xlvii, 328.

3 *Ibid.*, 1922, lxiii, 434.

4 *Ibid.*, 1925, lxxi, 553.

Numerous experiments were undertaken in which large doses of heparin—5 to 60 mg. per kilo of body weight—were injected intravenously in normal dogs and rabbits. In no instance was there any demonstrable gross disturbance of any body function.

It occurred to us, however, that the effects of repeated injections should be investigated. For this purpose, 20 mg. per cc. of heparin were dissolved in Ringer solution and injected into the peritoneal cavity of adult healthy guinea pigs, in relatively large doses, 20 to 60 mg. In a few instances symptoms characterized by prostration and weakness occurred within a few minutes after the first injection. After periods varying from 2 to 3 weeks, transcutaneous and intracutaneous tests were made with a similar solution of heparin, all of which proved negative. A second injection of a comparable dose of heparin was now made. In most cases this proved entirely negative, but in a few instances there occurred symptoms suggestive of anaphylaxis.

These results, together with those noted following the first injection, suggested two possibilities: 1. There might be a protein fraction in the heparin that sensitized certain more susceptible animals. 2. The heparin possessed toxic properties of undetermined nature.

Subsequent employment of fresh lots of heparin uniformly failed on both first and second injections to produce either toxic or anaphylactic symptoms, so that it is possible that the particular lot first used had not been satisfactorily purified.

Several lots of material from various stages in the purification of heparin were secured.† Some of these were highly toxic, both first and second injections producing immediate symptoms from which all the animals recovered. The most highly purified material, like heparin, gave entirely negative results after both first and second injections.

In another series, heparin was injected, and after 3 weeks relatively large doses of normal horse serum were injected intraperitoneally with negative results.

In still another series a sensitizing dose of horse serum was injected, and after 3 weeks a large dose of heparin. These were also negative.

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† These fractions, as well as part of the pure heparin, were generously supplied by the manufacturers, Messrs. Hynson, Westcott, and Dunning, Baltimore, Md.

The net result of 58 experiments was a complete failure to demonstrate by cutaneous or systemic reactions any antigenic properties for heparin.

The power of heparin to *modify* the antigenic action of horse serum is still under investigation.

3105

An attempt to locate cells of kinaesthetic sensibility in extraocular eye muscles.

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[*From the Department of Anatomy, Johns Hopkins University, Baltimore, Md.*]

Experimental work on dogs, cats, and pigs has thus far yielded the following positive findings. The cells of the III, IV, and VI cranial nuclei of the dog and cat can be separated into two distinct sizes, hitherto unrecognized, both having "motor" tigroid substance, and being in general diffusely intermingled throughout the nuclei. The smaller cells do not preponderate in any portion of the III, IV, or VI nuclei, nor in any of the subnuclei of the III, except Perlia's median nucleus which, in the dog, is made up entirely of the smaller cells. In the dog, the proportion of the sizes of the cells by actual count correlates roughly with the sizes of fibers in the peripheral trunks, more especially in the case of the third cranial pair. There is in the dog an excess of cells in the central nuclei of the extraocular muscles over the number of fibers in the peripheral homologous trunks, roughly, 30 to 40 per cent for the Nn. III and IV, and 10 per cent for the N. VI. Adequate clumps of cells along the peripheral nerves for the mediation of kinaesthetic sense of the extraocular muscles have not been demonstrated. The accumulated evidence of experiments with degenerations thus far indicates the possibility that the smaller cells in the central nuclei may mediate kinaesthetic sense of the extraocular muscles. The problem is being attacked further.

3106

Changes occurring in mammalian muscle immediately after death.

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In the numerous observations made in recent years on the chemical changes which set in after death in muscle, little attention has been paid to those which occur in mammalian muscle during the first few minutes after the cessation of blood flow. The increase which occurs in free phosphorus after two hours incubation of muscle juice or chopped muscle, and from which the so-called lactacidogen is calculated, has not been clearly linked up with the changes which take place in glycogen, lactic acid and free sugar. In the theories of Meyerhof,[1] A. V. Hill,[2] and their co-workers,[3] with regard to muscular contraction, it is considered that lactic acid is very rapidly formed from glycogen. But this cannot occur directly; there must be some intermediate carbohydrate, and it was to obtain evidence of this that present investigation was undertaken. That such a substance may be formed in muscle during the action of insulin is suggested by various observers.[4]

The muscles were rapidly dissected from one hind limb of a rabbit immediately after stunning, and frozen to a brittle mass with liquid air. After thorough pulverization in an iron mortar, quantities of from 3 to 5 gm. each of the powdered muscle were placed in weighed vacuum tubes. After all the tubes had again been weighed, equal volumes of 0.9 per cent NaCl solution were added to each, and they were evacuated and well shaken at room temperature. At intervals of 3 to 5 minutes tubes were then taken for the determination of glycogen, free sugar, total carbohydrate, free phosphorus and lactic acid. The results of a typical experiment are shown in curve form.

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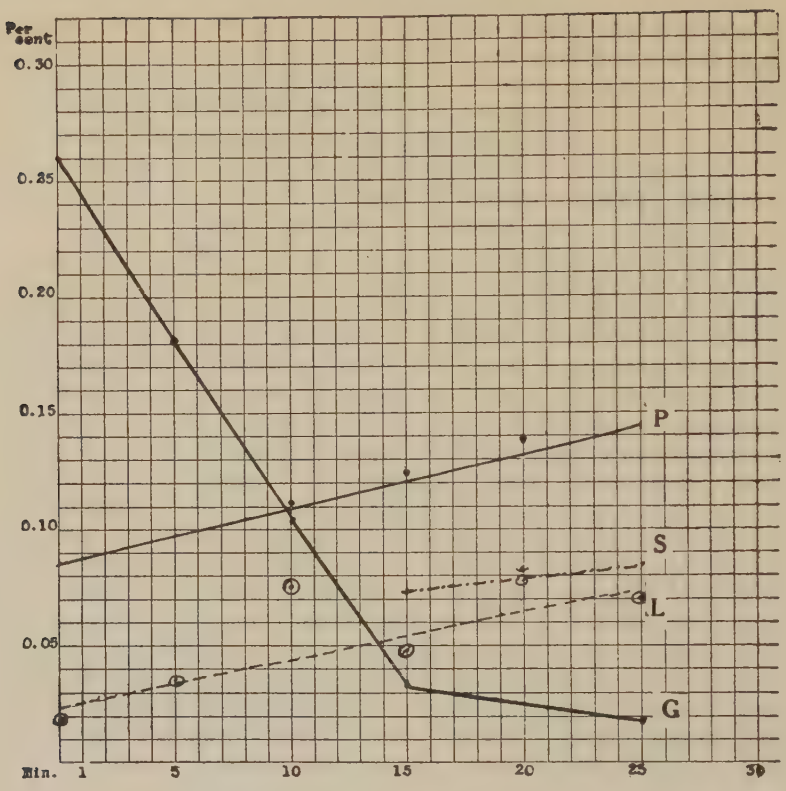
* Student of the National Research Council (Canada).

1 Meyerhof, O., "Chemical Dynamics of Life Phenomena," Lippincott, 1925.

2 Hill, A. V., "Muscular Activity," Williams & Wilkins, Balt., 1925.

3 Andrews, S., Beattie, F., and Milroy, T. H., *J. Physiol.*, 1924-25, lix, *Proc.* 13.

4 Embden, G., and Laquer, F., *Zeit. f. Phys. Chem.*, 1914, xciii, 94. *Ibid.*, 1917, xcviii, 181; *ibid.*, 1921, cxiii, 1.



The free sugar in this particular experiment was that found in the Schenck filtrate, but the results are the same in numerous others in which the sugar was dissolved out from the muscle by 80 per cent alcohol.

The most striking result is the very rapid disappearance of glycogen. This has been found in numerous experiments to occur invariably, and it indicates among other things that extreme care must be taken when determining the glycogen content of mammalian muscle, to work with great speed. In similarly treated liver, glycogen disappears very much more slowly, by a process which is evidently of an entirely different nature from that occurring in muscle. The lactic acid and the free phosphorus and the sugar increase slowly and steadily, from the moment of thawing, so that rapid disappearance of glycogen must be due to its conversion into some intermediary form of carbohydrate (lower dextrin?), and the process by which phos-

phoric and lactic acids are derived from this must be one that is quite independent of that by which the glycogen itself is broken down.

3107

Experimental relaxation of the pubic ligament of the guinea pig.

FREDERICK L. HISAW. (Introduced by Edgar Allen).

[*From the Zoological Laboratory, University of Wisconsin, Madison, Wis.*]

The extraordinary separation of the pubic bones of the guinea pig in late pregnancy has been recorded by several investigators. Todd[1] has made the most recent contribution to this subject and has thoroughly discussed previous research as well as described the gradual changes that take place at the symphysis during normal pregnancy and the subsequent return to the post-parturient condition typical of multiparous females. The writer has been interested in this phenomena for the last four years, chiefly from the standpoint of its physiological explanation and possible correlation with a similar condition which has been studied in the pocket gopher.[2] In the pocket gopher the pubic bones are resorbed at the symphysis before pregnancy occurs, and the reaction is governed by the ovary, while in the guinea pig relaxation of the pubis occurs during pregnancy and little or no bone is resorbed, but the connective tissue at the symphysis is greatly increased, allowing a marked separation of the bones.

It has been possible to produce changes in the pubic ligament of virgin guinea pigs by experimental procedure and these are apparently identical with those occurring normally during pregnancy. If 2 cc. of blood serum of pregnant rabbits or guinea pigs are injected subcutaneously into virgin guinea pigs during early post oestrum a noticeable relaxation of the pubic ligament can be discerned within six to eight hours by movements at the

[1] Todd, T. W., *Am. J. Anat.*, 1923, xxxi, 345-357.

[2] Hisaw, F. L., *J. Exp. Zool.*, 1925, xlii, 411-441.

pubis. These movements gradually become more pronounced during the next eighteen hours and the ligament may not return to its normal condition for two or three days. The blood of males and nonpregnant females does not bring about this reaction, and the blood of parturient females loses its effectiveness very noticeably within the first eight hours, negative results being usually obtained from blood drawn 24 hours after the young are born. The blood of all pregnant animals does not possess the ability to bring about this reaction. Serum prepared from the blood of pregnant cats, dogs, and rats gave entirely negative results when injected into virgin guinea pigs that responded positively to rabbit serum.

These results, while interesting, do not throw light on the source of this active substance and an attempt has been made to locate the organ or organs responsible for its production. The fact that the pubic ligament seems to be more easily influenced when pregnant serum is administered at a time close to oestrum, apparently indicates that the ovary plays some part in the process, but the relaxation and connective tissue multiplication at the symphysis does not cease after pregnant animals are spayed. Also the injection of saline extracts of dessicated whole ovaries gave entirely negative results while the injection of liquor folliculi gave only an occasional positive reaction. It is also possible to inject the extracted follicular hormone and produce artificial oestrum of long duration without noticeably effecting the pubic ligament, but if pregnant rabbit serum is then injected positive results are obtained quite readily, showing that the animals were in the proper physiological condition to respond. These observations indicate that the ovary may be of some importance but is not entirely responsible for a positive reaction.

It is also possible to produce relaxation of the pubic ligament by the injection of other materials. Positive results were obtained quite readily through the injection of saline extracts of rabbit placenta and whole amniotic liquor, but saline extracts of fetuses gave only negative results. These observations when considered with the fact that the blood of pregnant rabbits loses its relaxative properties within a short time after birth of young, and that relaxation is produced more easily during early post oestrum, point toward the conclusion that the pubic ligament, through the action of the ovary, is put in a physiological condition favorable for re-

sponding to relaxative materials secreted by the foetal membranes. It is appreciated, however, that the data at hand are not extensive enough to form a sound basis for far-reaching conclusions but seem worthy of a brief preliminary report.

3108

The behaviour of caramelised carbohydrates.

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A recent note in the PROCEEDINGS by Deuel, Mandel and Waddell[1] leads us to report one of a series of experiments which we undertook some time ago, using a preparation of caramelized oatmeal with normal diabetic patients as suggested by Grafe.[2] Grafe had used caramel as a substitute for ordinary carbohydrates as far back as 1911.[3] It was found, however, that its use was liable to produce diarrhea.

The preparation which we used was Satrose, made by Messrs. Schering. It is said to contain nitrogen 2 per cent; fat 2.3 per cent; cellulose 8 per cent; ash 2 per cent, and carbohydrate 75 to 80 per cent. It is a brown powder with the taste of burnt brown paper. Although we gave it in the way suggested by the makers, we were unable to induce many of our patients to take it. This, combined with the fact that we were not able to make out any notable effect on the metabolism, lead us to abandon further work with it. From the standpoint of practical dietetics with the diabetic it does not appear to possess any advantage.

We give the results of administering 50 grams of Satrose and 50 grams of glucose to the same diabetic patient in the post-absorptive state.

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* Assisted by a grant from the Medical Research Council.

1 Deuel, H. J., Mandel, A. R., and Waddell, S. F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 431.

2 Grafe, E., *Deutsch. Arch. für klin. Med.*, 1923, cxliii, 1; see also Grafe, E., and Otto-Martiensen, *ibid.*, p. 87, and Magin, H. and Turban, K., *ibid.*, p. 97.

3 Grafe, E., *Deutsch. Arch. für klin. Med.*, 1914, cxvi, 437.

A. L., male. 23.10.24. Satrose, 50 grams, at 10.38 A. M.

Time	R. Q.	B. M. R. per cent	Blood sugar per cent
10:18	0.80	+12	0.132
10:58	0.77	+11	0.148
11:40	0.81	+11	0.194
12:35	0.82	+18	0.194

No glucose was excreted in the urine during this experiment.

The same. 28.10.24. Glucose, 50 grams, at 10.07 A. M.

Time	R. Q.	B. M. R. per cent	Blood sugar per cent	Glucose excreted grams
9:45	0.80	—12	0.118	
10:30	0.81	+ 1	0.146	
11:20	0.79	+ 5	0.240	0.4
12:00	0.78	+ 1	0.298	
12:35	0.82	— 4	0.260	0.8

3109

On the R-T interval in experimental coronary occlusion.

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In 1918 Smith[1] published electrocardiographic tracings following ligation of coronary vessels in the dog. In one tracing, five minutes after tying the circumflex branch of the left coronary artery, there occurred, among other changes, marked elevation of the T-wave, with the T-wave originating on the down stroke of the R-wave, when the latter had reached about one-half the distance to the base line.

In 1920 Pardee[2] published a similar electrocardiogram obtained from a patient four hours after an obstruction of a coronary vessel.

[1] Smith, Fred M., *Arch. Int. Med.*, 1918, xxii, 8.

[2] Pardee, H. E. B., *Arch. Int. Med.*, 1920, xxvi, 245.

More recently, Rothschild, Mann, and Oppenheimer[3] renewed the interest in the peculiar change of the R-T interval as an early diagnostic sign of coronary obstruction. They reported observations on four patients shortly after acute coronary occlusions in which among the first changes in the electrocardiogram there was the characteristic elevation of the R-T interval above the base line.

In 1909 Eppinger and Rothberger[4] observed in dogs that partial or complete absence of the desecending limb of the R-wave was a relatively frequent and characteristic occurrence following the injection of silver nitrate solution into the muscle of the left ventricle whether at the base or apex. The T-wave became high and broad and originated either at the peak of the ascending limb of the R-wave or from the lower end of the descending limb which failed to reach the base line. They explained this effect on the basis of impaired contraction of the mass of circular muscle fibres in the structure of the left ventricle.

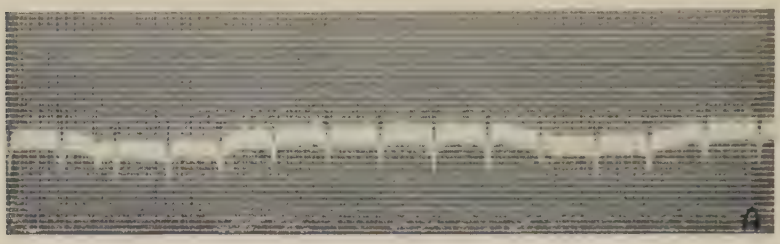
In a study of the action of digitalis in experimental coronary obstruction, one of us[5] made the observation that when the left coronary artery was ligated at the aorta in the cat, there occurred immobilization of the left ventricle, which also failed frequently to go into fibrillation, while the right side of the heart continued to beat after the ligature was applied and, as a terminal event, usually went into fibrillation. If the partial or the complete absence of the descending limb of the R-wave is caused by interference with contractility of the left ventricle, it seemed that the electrocardiographic change above described should result frequently from tying the left coronary artery at the aorta in the cat. This change was found in three of five experiments within two to five minutes after ligation of the entire left coronary artery at the aorta. Electrocardiograms were taken beginning before the ligation and continuing until death. The characteristic change in the R-T interval was absent in one instance in which a severe hemmorhage occurred and in another instance in which the heart went into ventricular fibrillation within one minute after the ligature was tied. In the course of another experiment only the anterior branch of the left coronary artery was tied.

[3] Rothschild, M. A., Mann, H., and Oppenheimer, B. S., Proc. Soc. Exp. Biol. and Med., 1926, xxiii, 253.

[4] Eppinger, H., and Rothberger, C. J., *Wien. klin. Wchnschr.*, 1909, No. 2, 1091.

[5] Gold, H., *Arch. Int. Med.*, 1925, xxxv, 482.

This also resulted in the characteristic change in the R-T interval. These experiments tend to confirm the view of Eppinger and Rothberger[4] that the failure of the R-wave to descend to the base line is due to impaired contractility of the left ventricle. The immobilization of the left ventricle is almost certainly greater within minutes to hours after the ligation than subse-



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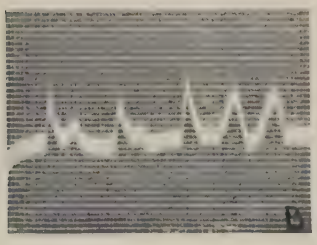

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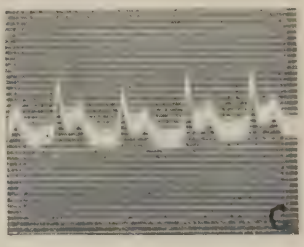

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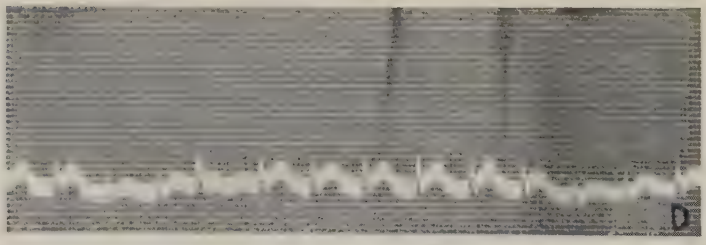

D

FIG. 1.

Showing electrocardiograms of experiment 3. Lead 1 only was taken. Section A is tracing before ligation of left coronary artery at the aorta. Section B is tracing 4 minutes after ligature was tied. Section C is tracing 6 minutes after ligation. After the heart went into ventricular fibrillation the ligature was removed. After slight massage the heart resumed its beat. Section D shows partial recovery after removal of the ligature.

quently and the reappearance of the normal descending limb of the R-wave may indicate the establishment of compensatory circulation.

As already indicated, the elevation of the R-T interval above the base line is not specific for coronary obstruction. De Boer[6] and others have recorded similar electrocardiograms obtained from the frog's heart under various experimental conditions. Similar electrocardiograms have been obtained in animals under diminished oxygen supply, also after the injection of various drugs.

[6] de Boer, S., *Am. J. Physiol.*, 1925, lxxiv, 158.

Peking Branch

Peking Union Medical College, March 30, 1926.

3110

Observations on the "reversed" uterine horn of the rabbit.

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[*From the Department of Physiology, Peking Union Medical College, Peking, China.*]

It is universally believed that the ciliated epithelium of the oviducts and uterus of mammals play the chief role in the transport of the ovum from ovary to uterine cavity. A few authors, however, seem to regard the contractions of the uterine muscle as being more important.

The above belief is based on two facts, (1) the direction of the ciliary movement being vaginal-ward and (2) the appearance of small particles in the uterus or vagina, after being introduced into the general peritoneal cavity.

In attempting to establish the corollary, namely that if the direction of the cilia be reversed, the ovum could not progress beyond the part reversed, we have found that the reversal of the uterus offers no impediment to the passage of the ovum.

The method employed was to divide the uterine horn (of rabbits) on each side, in two places 2.5 to 3.0 cm. apart and to reunite the cut ends after reversing each segment. The animals were then allowed to heal (a laparotomy being performed in some cases to determine the condition of the anastomosis) and were later mated.

Six animals thus experimented upon have all become pregnant. Three of the animals were subjected to observation before term, the remainder were left to complete their pregnancy.

It was found that implantation occurred most frequently within the reversed segment and in one instance *in the portion of the uterus between the segment and the cervex uteri.* It is thus es-

tablished, that an ovum may travel across two anastomoses apparently against an opposing ciliary movement.

If ciliary action is still responsible, it would mean that the direction of ciliary movement is capable of change; whether this is the case or not we have not yet had the opportunity to observe. Direct observation of the normal uterine mucosa shows ciliary movement mostly vaginal-ward but in part in a transverse and even in an ovarian direction.

The possibility of motor activity being responsible for the transport of the ovum has been subjected to some preliminary tests. Thus in the normal non-pregnant uterus, contractions may be conducted both up and down the tube, there being a slight polarity favoring vaginal-ward conduction. In the pregnant uterus, the polarity appears to be more marked. In the uterus with a reversed segment, a wave of contraction does not seem able to be conducted across an anastomosis in any direction, while the segment exhibits its inherent polarity, thus conducting more readily in the "reverse" direction. The possibility of conduction in either direction, however, cannot be excluded, although the failure to conduct across an anastomosis (in rabbits operated under six months) is definite.

In the cases here described, the ovum has already traversed the narrow and poorly muscular oviducts before reaching the uterine horn.

Further, a small portion (about 1 cm. in length) of the uppermost part of the uterus remains as in the normal, and it would seem that it is the rhythmic contractions of this small but normally situated part of the uterus which force a mucous mass (it is doubtful if so small a body of the ovum could itself be propelled along by the peristaltic activity of the uterus) containing the ovum progressively towards the vagina.

3111

Note on the secreted concentration of HCL in the gastric juice.

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[*From the Department of Physiology, Peking Union Medical College, Peking, China.*]

It has been shown[1] that the secreted concentration of HCl (*i. e.,* the concentration of acid as it is secreted by the cell) may *not* vary between 0 and a concentration higher than the observed maximum concentration (about 0.6 per cent), but may vary between a certain value (at least 0.29 per cent) and the latter, if it does so at all.

The restriction of the limits of variation strongly supports Pavlov's contention, namely that HCl is secreted at a constant concentration. If this be true, it should be possible to test the validity of the assumption by observing the factor which converts the amount (weight) of HCl into volume (cc.) of acid secreted. If the factor behaves as a constant, the concentration must obviously be invariable.

Employing simultaneous equations of the following form,

$$hx + py + m = c$$

(x and y are factors for converting known HCl (h) and pepsin (p) values in terms of cc. (c), and m is the volume of mucus) taken from two successive observations over an equal period, in any one Pavlov- or Heidenhain-pouch dog, the following values of x were obtained:

0.15 (3), 0.16 (2), 0.17 (7), 0.18 (6), 0.19 (7),
0.20 (6), 0.21 (4), 0.22 (2), 0.23 (2).

The mean of $x = 0.19$ and the S. D. $= 0.02$. (The figures in brackets give the frequency of each observation in the series.)

It will be seen that x is relatively constant, probably as constant as can be expected from the methods available for the determination of pepsin and mucus.

The values for y, however, varied greatly (0.0001 to 0.14) and gave extremely low volumes for pepsin. If, therefore, pepsin be neglected and the Cl not accounted for by acid (*i. e.,* total Cl-HCl

[1] Lim, R. K. S., *Am. J. Physiol.*, 1924, lxix, 318.

Cl) be considered to be NaCl which has diffused through the gastric mucosa as an isotonic solution, and if this volume of non-acid fluid replaces py in the above formula, the solution of the simple equation gives the following values of x:

0.12 (1), 0.13 (1), 0.15 (2), 0.16 (2), 0.17 (4).
The mean of $x = 0.16$; the S. D. $= 0.02$.

According to the first value of x (0.19) the concentration of HCl as secreted by the cell would be 0.53 per cent, and according to the second value (0.16), it would be 0.63 per cent. These figures are suggestive when compared with the figure usually given as the maximum HCl concentration of the total gastric juice.

3112

The influence of lymphocytes on peptic digestion.

H. C. HOU. (Introduced by R. K. S. Lim).

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It has been demonstrated[1] that polymorphonuclear leucocoytes neither activate nor contribute to the proteolytic power of pepsin when added in quantities (100,000 to 500,000 per cc.) commonly occurring in the gastric juice of dogs. It has now been found that lymphocytes behave in a similar manner, (see Tables I and II). Lymphocytes (lymphocytes 93 per cent, monocytes 4 per cent, polymorphs. 3 per cent) from the dog's thoracic duct lymph, were, after repeated washings in saline, suspended in distilled water and immediately added either to dog's gastric juice or to known dilutions of Merck's pepsin and the whole adjusted to constant volume and acidity. The ferment activity was estimated by Mett's method.

TABLE I.

Pepsin concentration	0	0.001	0.01	0.03	0.10	0.35	0.6
Control. No lymphocytes	0	0	0.6	1.3	2.4	3.6	4.2
150,000 lymphocytes	0	0	0.6	1.3	2.3	3.6	4.2

[1] Hou, H. C., *Am. J. Physiol.*, 1926 (in press).

TABLE II.

No. of lymphocytes added	0	150,000	300,000	400,000	450,000	800,000
No pepsin	—	0	0	—	0	0
Merck's pepsin 0.35 per cent	3.6	3.6	3.5	—	—	3.3
Dog gastric juice (unfilt'd)	2.0	1.9	—	1.9	—	1.8
Dog gastric juice (filtered)	1.9	1.9	1.8	1.8	1.7	1.7

The results are given in mm.

These results do not lend support to the theory[2, 3] that the lymphocytes play a role in the peptic digestion of gastric juice.

3113

Effect on the eye of instillations of a ten per cent solution of pseudo-ephedrine.

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The alkaloid pseudophedrine is the dextroisomer of ephedrine. The salt used in our experiments, as prepared by Chou,[1] was the hydrochloride ($C_{10}H_{15}ON.HCl$) with a melting point of 179 to 181° C, and an optical rotation of $(\alpha)\ {}_D^{22°}$ C. + 58.75. It was very soluble in water and alcohol.

Experimental tests with a 10 per cent solution of pseudoephedrine were made on the eyes of thirteen individuals. The object of the tests was to determine what effect, if any, the drug has upon the acuity of vision, the sensitivity of the ocular mucous membrane, the intra-ocular tension, the pupil, the range of accommodation, the near-point of convergence, or in producing any other symptoms. subjective or objective. A record was first made

[2] Ohno, R., *Mitteil a. d. med. Fakultät d. Kaiserl.* Kyushu Universitat, 1924, lx, 307.

[3] Pavlovsky, A. J., *Semana med.*, 1920, xxvii, 398.

[1] Chou, T. Q., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 618.

of each subject's normal function. One drop of the solution was then instilled into each eye every two minutes for five doses. Subsequent observations were made at intervals of ten or fifteen minutes for a period of several hours.

RESULTS.

1. Vision: Six subjects had a slight diminution of vision, probably due to a moderate but uniform edema of the epithelial cells of the cornea which produced the effect of looking through a faint mist. One subject's vision was slightly increased, due possibly in his case to a spasm of the ciliary muscle, he being a hyperope. The remaining six subjects showed no change in visual acuity. On the whole the effect on acuity of vision may be considered as negative.

2. Anesthesia or hyperesthesia: There was neither loss nor increase of sensitivity of the cornea and conjunctiva.

3. Intra-ocular tension: A Schiötz tenometer, which measures the tension in millimeters of mercury, was used. No effect was obtained in the subjects observed. This result is different from that obtained with adrenalin or epinine which lower the intra-ocular tension. This test was not made upon all the subjects, since the test in itself was unpleasant. Furthermore, it was necessary, every time the tension was taken, to produce a temporary anesthesia of the cornea by instillations of holocain.

4. Mydriasis: Eight subjects showed no change in the size of the pupils. Five developed a partial dilatation of the pupils twenty to thirty minutes after the first instillation. The maximum dilatation that was produced, occurred forty to sixty minutes after the instillations were begun, and from then on the mydriasis slowly subsided. The increase in the size of the pupil varied from one to four millimeters. The mydriasis lasted from one to six hours.

5. Range of accommodation and near-point of convergence: Four subjects showed no change. One developed a moderate spasm of accommodation of three hours' duration. The remaining eight subjects showed either a slight or a moderate decrease in the power of accommodation, which was first apparent about thirty minutes after instillation. The maximum decrease was recorded at about the end of the first hour. The loss of accommodation varied from one-half to three dioptres and lasted from two to six hours. The average loss was about one dioptre. A

slight recession of the near-point of convergence occurred in association with the loss in the power of accommodation.

6. Other symptoms: a. Subjective: All the subjects complained of an immediate smarting and burning sensation produced by the instillations. The last drop caused as much discomfort as did the first, confirming the direct test that no anesthesia was developed.

All developed an intense lacrymation, which lasted for about twenty minutes.

All noticed a bitter taste like that of homatropine hydrochloride. Several spoke of a distinct sensation of distension of the eyeball, although tests for tesnion proved negative.

Several complained of a dull frontal headache which came on during the course of the second hour and lasted for three or four hours; one of these subjects developed nausea.

Several complained of a dryness of the conjunctiva, as well as of the skin of the eyelids, after the first thirty minutes.

b. Objective: All the subjects developed a marked congestion of the superficial vessels of the palpebral and bulbar conjunctiva, which lasted for about twenty or thirty minutes. The appearance of the conjunctiva then became normal, but was not followed by a blanching. No proptosis of the eyeball nor widening of the palpebral fissure was observed.

SUMMARY.

1. Pseudoephedrine in a 10 per cent solution is an uncertain mydriatic and cycloplegic. The age of the individual does not seem to be a factor. There does exist, however, an individual difference, probably due to a variation in the rate of the absorption of the drug.

2. The action of pseudoephedrine upon the eye is like that of homatropine, rather than like adrenalin, which it closely resembles chemically.

3. In 10 per cent solution pseudoephdrine is slightly toxic when instilled into the eye.

4. There is no clinical evidence that pseuodephedrine contracts the blood vessels as does ephedrine which confirms the laboratory studies upon frogs performed by M. Fujii.[2]

5. Pseudoephedrine in a 10 per cent solution apparently has

[2] Fujii, M., *Manshu Igaku (J. Oriental Med.)*, 1925, iii, 1.

no place in the treatment and examination of ophthalmic diseases. A stronger solution would undoubtedly be a more active mydriatic and cycloplegic but would be too toxic to justify its use.

6. The results of these experiments do not wholly agree with those of Lewin-Guillery.[3]

3114

Some similarities between the dystentery amoeba of the monkey and of man.*

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The dysentery amoeba of the monkey and that of man are so similar in structure that some regard them as probably being the same species, though other investigators have given them different species names. In an attempt to procure experimental evidence on this question two monkeys found to be amoeba-free were fed cysts of *E. dysenteriae* of man. Examination of the feces during a period of three months and of histological sections following autopsy showed the presence of cysts and trophozoites of *E. dysenteriae,* morphologically and racially indistinguishable from the amoeba fed, thus indicating that the dysentery amoeba of man may be experimentally established in the monkey. Subsequently, two cats, six months and seven months of age respectively, found negative for protozoa by preliminary examination, were given rectal injections of monkey feces containing cysts of the dysentery-like amoebae of a naturally infected monkey, using the technique of Boeck and Drbohlav.[1] Two months later, autopsy of the cats showed the amoebic infection to be well established in the upper colon and cecum, where there was excessive mucous, pronounced hyperemia, and a distinct thickening of the gut wall. Trophozoites of amoebae, many of which contained red blood

[3] Lewin-Guillery, *Wirkungen von Arzneimitteln und Giften auf des Auge,* Berlin, 1913, S. 204.

* Contribution No. 74.

[1] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.,* 1925, v, 371-407, 4 pls.

corpuscles, were found in great numbers in these regions and histological sections showed necrosis of the mucosa and penetration of the amoeba into the tissue. Amoebae from the infected colon of the cats were planted into the L. E. S. medium of Boeck[1] and at the time of writing seventeen successful subcultures have been made on alternate days and the cultures show no signs of dying out.

If the cat is proved to be specifically susceptible to *E. dysenteriae,* then experimental infection of cats with the dysentery amoeba of the monkey lends strong evidence in favor of the species identity of these two amoebae. The present work and also that of Mello,[2] who reports dysentery in kittens produced by an experimental infection of active amoebae from acute dysentery in the monkey, both support this hypothesis. The experimental transfer of the dysentery amoeba of man to the monkey and the easy cultivation of the dysentery amoeba of the monkey in the same medium in which *E. dysenteriae* is cultivatable are additional evidence in favor of this species similarity.

[2] Mello, U., *Ann. d'Igiene,* 1923, xxxiii, 533-552, 2 pls.

Minnesota Branch

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3115

Distemper in the silver fox (Culpes vulpes).

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In a previous communication[1] there was reported the isolation of organisms belonging to the genus *Salmonella* from foxes dying of an epidemic disease. These organisms have been shown to be very pathogenic for foxes. When injected into healthy foxes a disease is consistently produced, death usually occurring in from 14 to 20 days. The organism has been isolated from the spleen of an animal killed 5 days after injection, but no gross pathological changes had yet occurred. In artificially infected foxes the pathology at necropsy is characterized by an intestinal inflammation and enlargement of the spleen, lungs usually being normal. The spleen is sometimes enlarged 20 times, by weight. The same picture has been produced by a direct injection of material from carcasses of ranch animals.

Further experimental work has been carried out in vaccination of ranch animals with these organisms, and further studies of disease occurring in these animals, using for transmission experiments foxes which also have been immunized with a *Salmonella* vaccine. An epidemic on a large ranch had previously been studied in which the picture of a *Salmonella* infection was the characteristic pathology. The organisms were isolated from many animals. Vaccination of the entire ranch of more than 500 animals appeared to control the epidemic, which was mainly among foxes 4 months old.

[1] Green, R. G., Proc. Soc. Exp. Biol. and Med., 1924, xxii, 546-548.

During the ensuing months there were a few scattered deaths, and 6 months later there was an increase in the number of deaths sufficient to indicate the presence of a mild epidemic. In the second epidemic quite a different picture was presented from the one previous. There were no external signs of disease. Some animals were found dead; others were found in convulsions, dying shortly afterward, and a few were sick for several days before death. In most cases necropsy showed no marked gross pathology. The lungs were normal and intestinal inflammation was not present. Extensive efforts to transmit the disease from a dead animal were unsuccessful, except in possibly one case. A few foxes were obtained before death and blood and nasal washings taken. Blood serum was diluted and filtered through Berkefeld N filters, as were also the nasal washings. By the use of such materials a similar condition has been produced in a few healthy animals under well controlled conditions. Filtered blood serum from these artificially infected animals has also proved infectious.

The foxes used in these transmission experiments had previously been vaccinated with a *Salmonella* vaccine. The experimentally infected foxes have shown the same general picture exhibited by animals dying on the ranch under conditions of natural infection. Pneumonia and intestinal lesions were absent. No organisms of any kind could be isolated from the blood, and tissues of the ranch animals from which the infective material was obtained, and all similar cultures from the artificially infected animals were likewise sterile. The transmission of a disease by the use of filtered material indicates a filterable virus as the infective and fatal agent in those animals showing the absence of any gross pathological findings at necropsy. It is, therefore, indicated that there are at least two pathogenic agents concerned in the epidemic diseases of foxes.

3116

Rats on diets high in phosphorous and low in calcium.

GRACE MEDES.

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It has been reported by a number of investigators[1, 2, 3] that rats become rachitic on diets high in P and low in Ca.

Two rats were kept on a normal diet (A) containing 302 mg. P and 414 mg. Ca per 100 gm. diet. Another group of two were given a diet (B) low in P (124 mg. P and 372 mg. Ca per 100 gm. diet). A third group of two were given a diet (C) containing 1044 mg. P and 19 mg. Ca per 100 gm. diet. After three weeks the rats were photographed by X-rays and ashed. The ash was analyzed for Ca, P and Mg. The X-ray photograph showed that the bones of the rats on low Ca, high P were normal, except for a slight osteoporosis. In marked contrast to those on normal diet and diet low in Ca, both rats which received diet (B) became rachitic.

The percentages of Ca and P in the ash of those on diet C were slightly less than in normal rats (♀) of their own age. The P/Ca ratio in the ash of the rats was normal (0.72). The rachitic index figured by McClendon's formula[4] was (A) 5.2, (B) 1.4, and (C) 2.0.

Diet	Sex	Av. age	Av. wt.	Ash of rat						
				Composition			Per cent body wt.			
				Ca	P	Mg	Ca	P	Mg	
		Das.	gm.	mg.	mg.	mg.				
A. Normal	♂	52	101	749	564	39	0.74	0.56	0.039	Normal
	♀	51	67	562	434	27	0.84	0.64	0.041	
B. Low P	♂	48	75	454	268	21	0.60	0.36	0.028	Severe rickets
	♀	48	73	320	195	23	0.44	0.27	0.032	
C. Low Ca, High P	♀	51	66	469	350	27	0.72	0.53	0.041	Decreased calcification. No rickets.

1 McCollum, E. V., and Simmonds, Nina, *Am. J. Hyg.*, 1922, i, 492.

2 Pappenheimer, A. M., McCann, G. F., and Zucker, T. F., *J. Exp. Med.*, 1922, xxxv, 447.

3 Kramer, B., (personal communication).

4 McClendon, J. F., *Am. J. Physiol.*, 1922, lxi, 373.

3117

Biochemistry of plant diseases. VII. Correlation between skin texture and flesh texture in plum varieties.*

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In a previous paper[1] data were presented which led to the belief that the comparative resistance of certain plum varieties to brown rot is due to mechanical resistance to the entrance of the fungus. The more resistant varieties had a tougher skin and a firmer flesh; and, at the same time, they had a higher crude fiber content. Preliminary calculations of correlation by rank between skin and flesh texture indicated that the two factors, tough skin and firm flesh, varied together. If this premise could be proved to hold generally, it would be of value in further work on plums, since only one of the two factors would need to be measured.

A considerable number of measurements of these two constants are now at hand. They cover the three seasons of 1923, 1924, and 1925; they involve over 45 varieties of plums grown at the Fruit Breeding Farm of the University of Minnesota; and they have been taken at all stages of ripeness. These data are presented in the accompanying table.

Each sample, representing a variety at a particular stage of ripeness, consisted of from 4 to 6 plums. The puncture test on the skin and the penetration test on the flesh, as described in the previous paper, were made from 3 to 6 times on each plum. The

Correlation between Skin Texture and Flesh Texture in Plums.

Season	Material	Number of varieties	Stages of Ripeness	Value of n	Coefficient
1923	Lots	11	I thru VI	66	$-.788 \pm .032$
1924	Individuals	5	I thru V	105	$-.415 \pm .055$
1925	Individuals	39	III and IV	243	$-.418 \pm .035$
1925	Individuals	23	III	94	$-.381 \pm .059$
1925	Individuals	25	IV	123	$-.322 \pm .054$
1925	Lots	39	III and IV	50	$-.445 \pm .076$

* Published with the approval of the Director, as paper No. 611, Journal Series, Minnesota Agricultural Experiment Station.

[1] Willaman, J. J., Pervier, and Triebold, *Bot. Gaz.*, 1925, lxxx, 121.

values used in the calculations were sometimes the averages of the readings on the individual plums, at other times the averages for the lot. In the 1925 material stages III and IV were used both separately and together. In the other cases all stages were included in one calculation.

It is apparent from the magnitude of the coefficients that a very significant negative correlation exists between the values for the two constants. It should be mentioned that a tough skin gives a high puncture value and a firm flesh a low penetration value. Hence, a negative sign to the cofficients indicates that these two mechanical factors in plums vary together. This relation seems to hold consistently for different seasons, for different varieties, and for different stages of ripeness. It is believed that sufficient evidence is now at hand to warrant the use of the skin test alone in studying the brown rot problem in plums.

3118

Effect of oxygen and carbon dioxide concentration on inhibition of respiration and photosynthesis by KCN.

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The present paper briefly presents certain facts which extend present knowledge regarding the effects of some of the following external conditions on the velocity of respiration and photosynthesis in the marine kelp *Nereocystis*. Some of the facts do apply, and the others may apply to respiration and photosynthesis of plants in general.

Uniform strips 1 x 10 cm. were cut from the frond of the kelp. The oxygen exchange in respiration and photosynthesis was determined by Winkler's method. Specially made bottles of 25 cc. volume were used. One strip was placed in each bottle for a determination. Three duplicate bottles with strips were used for the same concentration of oxygen, cyanide, etc. Each figure in the tables is, therefore, the average of three duplicate, simulta-

neous determinations. During the tests the experimental bottles were suitably immersed in the open sea, which afforded a constant temperature to within ±0.5° C. during any one experiment. Light intensity was the same in all the tests of any one experiment, but varied more or less from one experiment to another. Suitable control experiments showed that the effects reported are not due to differences in hydrogen ion concentration.

Effect of oxygen concentration on the rate of respiration and photosynthesis. It is a familiar fact that increase in concentration of oxygen increases the rate of oxygen consumption in many kinds of plants. It is not such a generally recognized fact that increase in concentration of oxygen decreases the rate of photosynthesis. These two effects of oxygen concentration on *Nereocystis* are shown by the three experiments performed simultaneously, and given in Table I.

TABLE I.

Experiment	1	2	3
	O_2 in sea water cc. thio.	O_2 consumed respiration cc. thio.	O_2 produced photosynthesis cc. thio.
1	4.62	2.42	7.23
2	7.66	2.97	5.12
3	13.87	3.67	3.84

Column 1 shows the initial concentration of dissolved oxygen in sea water at which the rates of respiration and photosynthesis were tested. The rates of oxygen consumption at the given concentration of oxygen are shown in column 2. The corresponding rates of oxygen production by photosynthesis are shown in column 3. Note that high concentrations of oxygen increase the rate of respiration while the same initial concentrations of oxygen markedly retard photosynthesis. The experimental error in the determinations does not exceed 0.2 cc. thiosulfate equivalent of oxygen.

Effect of oxygen concentration on the rate of respiration in KCN. Table II is a summary of the results of three separate experiments which are not fully comparable with one another because they were performed at different times. Column 3 shows the rate of respiration in .000076 mol. KCN in per cent of the

TABLE II.

Experiment	1	2	3	4
	O_2 in sea water cc. thio.	O_2 in sea water cc. thio.	O_2 Respiration in KCN in per cent of normal	O_2 Respiration in KCN in per cent of normal
1	4.09	12.66	5%	49%
2	5.02	7.62	11%	69%
3	4.03	11.59	22%	76%

normal respiration in pure sea water, when the initial concentration of oxygen was that given in column 2. Similarly, column 4 shows the rate of respiration in the same concentration of KCN, in per cent of the normal respiration in sea water having the initial concentration of oxygen given in column 2. It is evident that as the concentration of dissolved oxygen is *increased* the magnitude of the inhibitory effect of cyanide is *decreased*. Ex-

TABLE III.

Column 1 shows (in cc. thiosulfate equivalent of oxygen produced) the normal rate of photosynthesis in pure sea water at air saturation. Column 4 shows the rate of photosynthesis in different concentrations of KCN (column 2) in sea water. Two tests were made at each concentration of KCN. To one of these were added 2 cc. or 3 cc. CO_2 saturated sea water column 3. Column 5 shows the rate of photosynthesis and degree of recovery of the same tissue in pure sea water 11 to 19 hours after the first test in KCN, and KCN and CO_2, column 4. Note that additions of CO_2 in experiments 3 and 4 protected the photosynthetic mechanism from injury by light when in the presence of KCN. Each number in columns 4 and 5 is the average of three duplicate tests.

Experiment	1	2	3	4	5	6
	Normal rate of photosynthesis	KCN mol.	cc. CO_2 saturated sea water added	1st test. Rate of photosynthesis. cc. thio.	2nd test. Rate of photosynthesis. cc. thio.	Condition of strip
1	7.37	$.76x10^{-5}$	0	3.95	9.59	All normal
			2 cc.	14.23	10.14	All normal
2	8.86	$.76x10^{-4}$	0	1.55	8.50	All normal
			2 cc.	8.00	7.58	All normal
3	9.73	$.38x10^{-2}$	0	.07	.26	All leached
			3 cc.	1.04	7.34	All normal
4	8.22	$.76x10^{-2}$	0	.86	.50	All leached
			3 cc.	.95	6.44	One leached

periments show that in appropriate concentrations of oxygen and cyanide the inhibitory effect of cyanide on the oxidations may be entirely wiped out.

Table III represents the condensed statements of the results of four experiments, as follows:

Effect of concentration of cyanide on the rate of photosynthesis. The normal rates of photosynthesis in pure sea water, in the four different experiments are given in column 1. The comparatively uniform rates in the different experiments show that external conditions varied within narrow limits from one experiment to the next. In column 4 it will be seen that the rates of photosynthesis in the corresponding concentrations of cyanide given in column 2, were 3.95, 1.55, .07, and .86 cc. thiosulfate equivalent of oxygen. As the concentration of cyanide increases the percentage inhibition increases until it becomes practically complete at $.38 \times 10^{-2}$ mol. KCN. This confirms the observations of Lund and Holt,[1] and the earlier ones by Warburg[2] on the green alga *Chlorella pyrenoidea.* It will be seen in column 5 that after exposure in light to $.76 \times 10^{-5}$ and $.76 \times 10^{-4}$ mol. KCN, complete recovery of the photosynthetic mechanism occurred. This is shown by the fact that the rate of photosynthesis in pure sea water after recovery was 9.59 cc. and 8.5 cc. respectively. In the two high concentrations of cyanide, $.38 \times 10^{-2}$ and $.76 \times 10^{-2}$ mol. inhibition was practically complete, but recovery did not occur in pure sea water. In fact the xanthophyll and the chlorophyll pigments leached out of the chloroplasts and the cells, leaving the tissue colorless and without turgor.

High concentrations of CO_2 protect the chloroplast and cell against the phototoxic action of cyanide. To show the effect of CO_2 on the phototoxic action of KCN, 2 cc. (experiments 1 and 2) and 3 cc. (experiments 3 and 4) of CO_2 saturated sea water were added to each of the duplicate sets of bottles containing the same concentrations of cyanide given in column 2. Note that the increased concentration of CO_2 removes completely the inhibitory effect of the lower concentrations of cyanide in experiments 1 and 2.

Recovery in pure sea water is complete in experiments 1 and 2, column 5. While 3 cc. of CO_2 saturated sea water in experi-

[1] Lund, E. J., and Holt, V., PROC. SOC. EXP. BIOL. AND MED., 1923, XX, 232.
[2] Warburg, Otto, *Biochem. Zeitschr.*, 1920, ciii, 188.

ments 3 and 4 did not prevent the nearly complete inhibition of photosynthesis by the high concentrations of cyanide, it did completely protect the photosynthetic mechanism against injury in $.38 \times 10^{-2}$ mol. KCN because recovery in pure sea water was practically complete and no bleaching of the pigments or loss of turgor occurred. Even in experiment 4 with a concentration of $.76 \times 10^{-2}$ the added CO_2 afforded almost complete protection except to one of the strips.

3119

Changes in the excretion of uric acid produced by experimental hepatic insufficiency.

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Destruction of uric acid, which is rapid and marked in the normal dog,[2] does not occur if the liver is entirely removed. Complete removal of the liver in the dog produces a very great increase in the uric acid content of the blood and tissues, and also in the urine. Uric acid injected into the dehepatized dog remains unchanged in the blood and tissues and is excreted unchanged in the urine. The destruction of uric acid in the dog seems to be entirely dependent on the presence of the liver,[1] since no uric acid is destroyed in the absence of the liver and no other means of influencing the destruction of uric acid has been demonstrated. Intravenous injection of standard amounts of uric acid into dogs with hepatic insufficiency is followed by a delay in the disappearance of the excess uric acid from the blood, and by an increase in the amount of uric acid excreted in the urine. Both the delay in the disappearance of the excess uric acid from the blood and the amount of uric acid appearing in the urine are greater, the greater the amount of damage or reduction of hepatic tissue. Two

[2] Folin, O., Berglund, H., and Derick, C., *J. Biol. Chem.*, 1924, lx, 361-471.

[1] Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1925, lxxii, 629-646.

objections should be raised to the use of injections of uric acid as a test of hepatic function. First, injections of large amounts of uric acid produce severe lesions in the kidneys and interfere with excretion; and second, lesions of the kidneys may produce retention of uric acid, although in the dog the presence or absence of the kidneys is without effect on the rate of disappearance of uric acid from the blood.

When a diet rich in purines is fed to the normal dog the excretion of uric acid may be measurably increased. Following an eighteen-hour fast, animals fed 175 gm. of fresh pancreas excrete from 80 to 160 mg. of uric acid during the twenty-four hours after feeding. The average amount of uric acid excreted by normal dogs on this diet is about 120 mg. Animals with definite hepatic damage show an increase in the amount of uric acid in the urine. Following ligation of the common bile duct the excretion of uric acid remains within normal limits for about four weeks. After the fourth week of obstructive jaundice the excretion of uric acid increases up to from 200 to 500 mg. of uric acid, following a diet of 175 gm. of fresh pancreas. There is no apparent relationship between the duration of obstructive jaundice and the excretion of uric acid, although the amount of uric acid excreted is roughly proportional to the estimated hepatic atrophy found on exploration or necropsy of animals with obstructive jaundice.

Animals with an Eck fistula also show a marked increase in the amount of uric acid excreted on a diet of pancreas. They excrete from 120 to 750 mg. of uric acid or an average of about 450 mg., the amount of uric acid excreted being roughly proportional to the amount of hepatic atrophy as estimated at exploration. However, there was no apparent relation between the duration of the fistula and the amount of uric acid excreted. Individual animals varied considerably with regard to the amount of uric acid excreted at different periods following the production of an Eck fistula, and some have approached normal after periods of rather high excretion of uric acid. The gross and microscopic appearance of the liver with an Eck fistula also varies considerably, and it appears that the amount of uric acid excreted increases with the damage of the liver.

Surgical removal of portions of the liver from animals with an Eck fistula is followed by only slight regeneration of hepatic tissue; thus we have been able permanently to reduce the

amount of hepatic tissue to only a small percentage of the normal. By careful dietary measures animals so treated may be maintained in apparently good condition for several years, and after a short time there seems to be but little change in the gross or microscopic appearance of the hepatic tissue. Following the ingestion of 175 gm. of fresh pancreas these animals excrete from 600 to 900 mg. of uric acid, or an average of about 700 mg. The amount of uric acid excreted was quite constant for the individual animals of this series, and increased in proportion to the reduction of hepatic tissue.

3120

The prenatal growth and natal involution of the human uterus.

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The human uterus undergoes a marked reduction in length and weight in the first few weeks following birth. This was first described by Lyubetski,[1] and later, independently, by Bayer[2] and by Conte.[3] This reduction takes place through hypoplasia and hypotrophy of the uterine muscle, together with a disappearance of the marked natal hyperemia of the organ. It is supposed to be caused by the withdrawal at birth of a hormone produced by the placenta, the ovary or the tissues of both of these structures, (Aschner,[4] Herrmann,[5] Fellner,[6,7] Frank,[8,9] and

[1] Lyubetski, N. S., Anatomical changes in the uterus in children. Diss. St. Petersburg, 1900.

[2] Bayer, H., *Deutsch. Arch. klin. Med.*, 1902, lxxiii, 422.

[3] Conte, G., *Atti Soc. Ital. d. Ostet e Ginecol.*, 1903, ix, 670.

[4] Aschner, B., *Arch. f. Gynäkol.*, 1913, xcix, 534.

[5] Herrmann, E., *Monatsschr. f. Geburtsh.*, 1915, xli, 1.

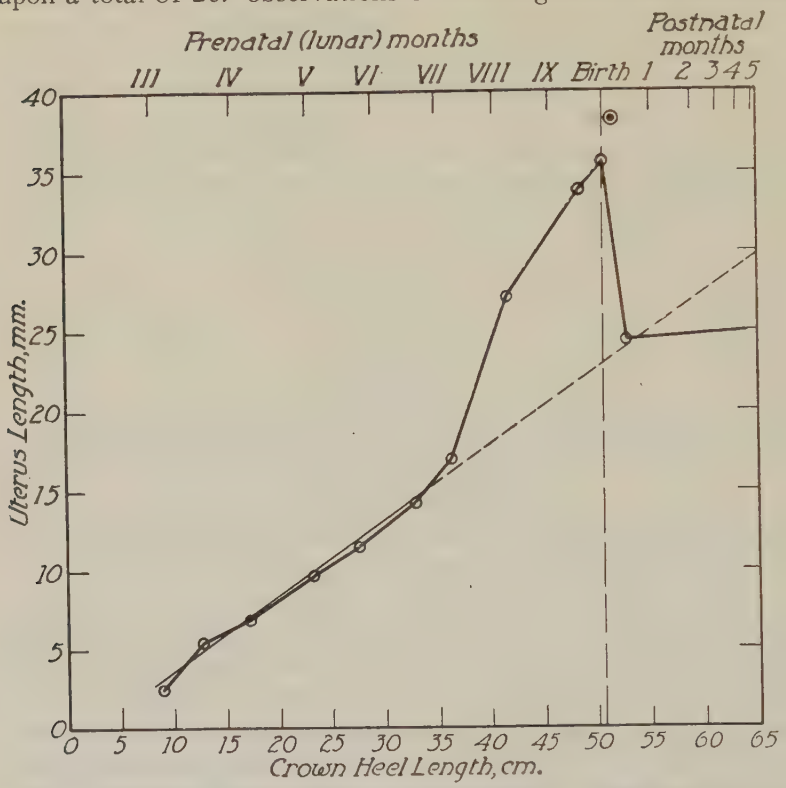
[6] Fellner, O. O. *Zentralbl. f. allg. Path. u. path. Anat.*, 1912, xxiii.

[7] Fellner, O. O., *Arch. f. Gynäkol.*, 1913, c, 641.

[8] Frank, R. T., and Rosenbloom, J., *Surg. Gynecol. and Obstet.*, 1915, xxi, 646.

[9] Frank, R. T., *ibid.*, 1917, xxv, 329.

others.) Little is known of the fetal growth of the uterus which precedes this natal reduction. The present study is based upon a total of 207 observations on the length of the uterus, 89



Graph illustrating lineal growth of human uterus in prenatal life and infancy. The uterus length (in mm.) is plotted against the total body-length (in cm.) and the computed age (in lunar months). The body-length is indicated on the base line and the computed age on the upper line of the graph. Age is computed according to the empirical formulae of Calkins and Scammon[10] and of Scammon.[11] Birth is represented by the vertical broken line of the graph. The circles, in the fetal period, represent the mean values for 5 cm. intervals of body-length. That at birth is the mean value for all newborn material. That at 15 postnatal days is the mean for 15 infants (of this average age) ranging from 3 to 27 days in age. The circled dot represents the mean value for 13 "postmature" newborn infants. The heavy solid line is the point-to-point curve of observed values. The light solid line is the computed curve for growth in uterus length, with respect to total body-length, up to 35 cm. crown-heel length. Its projection is represented by the oblique broken line extended from it.

[10] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 353.

[11] Scammon, R. E., *ibid.*, 1921, xix, 133.

of fetal uteri, 62 of uteri of infants stillborn or dying within 48 hours after birth, and 56 uteri of children over 2 days and under 1 year of age.

A graphic analysis of this material is shown in the accompanying figure, in which the mean uterus length for 5 cm. intervals of total or crown-heel body-length, is plotted against the total body-length (as indicated on the base-line scale), and the computed age, (as indicated on the upper scale of the graph). Starting with an average length of about 2.5 mm. in the 5 to 10 cm. interval of crown-heel length, the viscus grows at a slow and fairly constant absolute rate, with respect to the total body-length, until approximately 7 lunar months (about 35 cm. crown-heel length). At this stage the average length of the uterus is approximately 17 mm. The organ then enters a second phase, characterized by rapid lineal growth, and reaches a mean length of approximately 35 mm. at birth, or 10 lunar months.

Between birth and the third postnatal week the length of the uterus declines to 24.3 mm. This figure is based on the average of 15 cases, ranging in age from 3 to 27 days, with a mean age of 15 days. This is a loss of about 11 mm., or about one-third of the natal length. Thereafter there seems to be a slight increase in uterine length for the remainder of the first year, although this gain is so small that it is questionable.

The relation of uterus length to crown-heel length in the early part of prenatal life is approximately rectilinear. An empirical formula has been fitted to these data by the method of least squares, the expression being:

$$\text{U.L} = 0.4738\ \text{C.H.} - 1.106 \quad (1)$$

where "U.L." is the total length of the uterus in millimeters and "C.H" is the total or crown-heel length of the body in centimeters. The calculated values by this formula show a weighted mean deviation (without regard to sign of deviations) of 0.40 mm. from the corresponding observed mean values.

A straight line has also been fitted for the uterus length in the fetal period and may be represented by the expression:

$$\text{U.L.} = 1.3546\ \text{C.H.} - 31.045 \quad (2)$$

where the symbols are as in (1). The calculated values obtained by this formula show a weighted mean deviation, taken without

regard to sign, of 0.84 mm. from the corresponding observed mean values.

An estimate of the natal length of the uterus which would obtain if the structure continued to grow at the rate characteristic of the early part of prenatal life, may be made by projecting the line represented by formula (1) to 50.2 cm., which is the computed body-length at birth. This value is approximately 22.7 mm., whereas the observed value at birth is approximately 35 mm. Thus the uterus reaches the length at birth which is approximately one-third greater than that to be expected if the earlier growth-rate of the organ, with respect to body-length, were maintained in the latter part of the fetal period. If this line of early fetal growth is projected to approximately 53 mm., which is a computed mean length of the specimens representing the full post-natal involution of the uterus, it is seen that the length of the uterus, after its postnatal decrement, approximates the length of the organ, which might be predicted from its early prenatal growth. In other words, the uterus in the neonatal period declines in length to approximately the dimension which it would have attained had its early fetal growth rate remained until this time.

These figures indicate that there are two definite phases in lineal growth of the uterus in prenatal life. Until 7 months the organ shows a lineal increase, with respect to body-length, which is comparable to that of most lineal dimensions of the body and particularly to that of the pelvic dimensions. At about 7 lunar months it enters on a phase of augmented lineal growth. After birth the organ loses length until it assumes essentially the dimensions which it would have obtained had the early fetal growth rate remained unchanged. This suggests that the growth of the uterus in the latter fetal months consists of a substrate of typical fetal growth plus a secondary growth increment, which, presumably, is due to an extra stimulus furnished by a hormone of placental or possibly ovarian origin. After birth the organ loses this secondary increment but retains that resulting from the early fetal growth rate.

3121

A chemical study of cystine from kidney stones.*

ROSS AIKEN GORTNER and WALTER F. HOFFMAN.

[*From the Division of Agricultural Biochemistry, University of Minnesota, Minneapolis, Minn.*]

In 1923, Dr. C. E. Tennant[1] reported a surgical case in which 15 stones having a total weight of 73 grams were removed from a kidney. He noted that these stones were composed chiefly of cystine, which, upon purification, crystallized in hexagonal plates. Inasmuch as this material offered an unusual opportunity of again investigating the old question—is stone cystine identical in chemical composition with protein cystine—we secured, through the kindness of Dr. Tennant, a number of the kidney stones, and have analyzed them and prepared certain organic derivatives of the "stone" cystine. Our data, in summary, are:

1. 5.20 grams of the cystine stones yielded 4.84 grams, or 93 per cent of pure cystine crystallizing in typical hexagonal plates. Qualitative tests on the filtrate from the cystine crystallization indicated that small amounts of calcium and phosphate were present. Neuberg and Mayer[2] state that "protein" cystine crystallizes in hexagonal plates but "stone" cystine crytallizes in needles. We have found "protein" cystine to crystallize in the typical hexagonal plates, whereas our "isomeric"[3] cystine, prepared from "protein" cystine by long boiling with 20 per cent HCl crytallizes in microscopic needles.

2. The cystine crystals analyzed for 11.63 per cent nitrogen (theory 11.65 per cent) and 26.55 per cent sulfur (theory 26.67 per cent), and a 1 per cent solution in approximately 0.1 N HCl had a specific optical rotation of $[\alpha_D^{20}] = -242.6°$. Neuberg and Mayer[2] report —224° for the optical rotation of "protein" cystine and —206° for "stone" cystine. The value usually ac-

* Published with the approval of the Director as Paper No. 605, Journal Series, Minnesota Agricultural Experiment Station.

[1] Tennant, E. C., *J. Am. Med. Assn.*, 1923, lxxx, 305-7.

[2] Neuberg, C., and Mayer, P., *Z. physiol. Chem.*, 1905, xliv, 472-510.

[3] Hoffman, W. F., and Gortner, R. A., *J. Am. Chem. Soc.*, 1922, xliv, 341-360.

cepted[4] for "protein" cystine is $[\alpha_D] = -223°$ in HCl solution. Andrews[5] finds that the optical rotation is somewhat dependent upon the pH value. His values for a 1 per cent concentration of cystine range from $[\alpha_D^{29}] = -206.7°$ in 2.5 N HCl solution to $[\alpha_D^{29}] = -231°$ in 0.05 N HCl. Our value of $[\alpha_D^{20}] = -242.6°$ is decidedly higher than any value recorded in the literature for a solution of corresponding concentration. It would appear as though the usual methods for isolating cystine from protein material cause a slight racemization. This view agrees with our earlier findings.[3]

3. A microscopical examination of the di-hydrochloride showed the long needle crystals typical of the di-hydrochloride of "protein" cystine.

4. The di-benzoyl derivative (N found = 6.10 per cent, theory 6.25 per cent; S found = 14.11 per cent, theory 14.28 per cent) melted at 158° to 160° (uncor.) and crystallized in *diamond shaped plates.* The di-benzoyl derivative of "protein" cystine melts at 181° and crystallizes in long silky needles,[6, 7] while that of the "isomeric"[3] cystine melts at 110°[8] and crystallizes in diamond shaped plates. Neuberg and Mayer[2] report the melting point of the di-benzoyl derivatives of "stone" cystine as 157° to 159° (cor.) whereas that from "protein" cystine melts at 182° to 184° (cor.). Goldmann and Baumann[9] report a melting point for di-benzoyl cystine as 156° to 158°, but it is uncertain whether they were working with protein cystine or "stone" cystine. Apparently they were dealing with cystine derived from a case of cystinurea.

5. The phenylisocyanate crystallized in flat plates, M. P. 132° to 133° (uncor.) (N found = 11.64 per cent, theory 11.72 per cent; S found = 13.27 per cent, theory 13.39 per cent). Neuberg and Mayer[2] report "stone" cystine phenylisocyanate as melting at 170° to 172° (cor.) and "protein" cystine phenylisocyanate as melting at 160° (cor.). Shiple and Sherwin[10] also

[4] Abderhalden, E., "Biochemisches Handlexikon," Vol. 4, p. 657.

[5] Andrews, J. C., *J. Biol. Chem.*, 1925, lxv, 147-159.

[6] Brenzinger, K., *Z. physiol. Chem.*, 1892, xvi, 552-588.

[7] Gortner, R. A., and Hoffman, W. F., *J. Am. Chem. Soc.*, 1921, xliii, 2199-2202.

[8] Unpublished data. Data reported before the Organic Division of the American Chemical Society at the New York Meeting, September, 1921.

[9] Goldmann, E., and Baumann, E., *Z. physiol. Chem.*, 1888, xii, 244-261.

[10] Shiple, G. J., and Sherwin, C. P., *J. Biol. Chem.*, 1923, lv, 671-686.

report 160° (uncor.) for protein cystine. In our own work[8] we have found the following melting points for the pure phenylisocyanates: "protein" cystine M. P. = 148° to 149° (uncor.), "isomeric" cystine M. P. = 181° (uncor.). Both our "protein" cystine and "isomeric" cystine phenylisocyanates crystallized in long silky needles.

6. The phenyl hydantoin of the stone cystine was easily prepared from the phenylisocyanate derivative. It crystallized in needles, M. P. 112° (uncor.) (N found = 12.71 per cent, theory 12.67 per cent). Neuberg and Mayer[2] report that they were unable to prepare the phenyl hydantoin of "stone" cystine, whereas the corresponding derivative of "protein" cystine was easily prepared and melted at 110° (cor.). Shiple and Sherwin[10] and Patten[11] both report the melting point of "protein" cystine phenyl hydantoin at 117° (uncor.). We have found[8] "protein" cystine hydantoin to crystallize in fine needles and melt at 122° to 123° (uncor.), whereas the "isomeric" cystine derivative crytallizes in needles which melt at 166° (uncor.).

Conclusions. In the present instance some of the properties of the "stone" cystine are essentially identical with those which have been reported for "stone" cystine, in others with those reported for "protein" cystine, and in still others are apparently distinct from both. The only conclusion which can be drawn from the above conflicting observations, considered in the light of the cited literature, appears to be that cystine is an extremely labile compound and possibly occurs in more than one form, so that persons working with cystine are probably working with a mixture of substances and that this mixture varies in composition depending at least upon (1) the source of the biological material from which the cystine is prepared, and (2) the method of preparation which is used for the isolation and purification of this amino acid.

[11] Patten, A. J., *Z. physiol. Chem.*, 1903, xxxix, 351-355.

Massachusetts Branch

Harvard Medical School, April 13, 1926, and Massachusetts General Hospital, May 11, 1926.

3122

The velocity of venous blood to the right heart in man.

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[*From the Thorndike Memorial Laboratory, Boston City Hospital, and from the Department of Medicine of Harvard Medical School, Boston, Mass.*]

Our previous studies have shown the feasibility of measuring the velocity of blood flow in health and disease by injecting the active deposit of radium into one of the antecubital veins, and determining the time of arrival of the active deposit in the arterial vessels about the elbow of the other arm.

The path of the active deposit coursing through the body necessarily included the veins of the arm, the pulmonary circulation, and the artery of the other arm. The velocity measured was a somewhat complex expression, therefore, of the peripheral as well as of the central blood flow.

This report deals with an attempt to determine the separate velocities along these paths.

A detecting device was placed in a lead block. This block had a hole 5 cm. in diameter bored through its center. The radiations of radium C as it was injected into the veins and as it flowed towards the right heart could not penetrate the lead block and could therefore set up no disturbance in the detecting device. The hole of the lead block with the detector set into it, was placed over the right heart. When the active deposit reached the right heart, the emergent radiations easily traversed the air and tissues between the right heart and the detector, and set up characteristic

disturbances in the detecting device. These disturbances were amplified by three electrode vacuum tubes, and were finally registered automatically by a recording pen galvanometer. Knowing the time of injection of the active deposit and knowing the time of its arrival into the right heart, we have a measure of the velocity of the venous blood flow. Figure I is the record of a typical

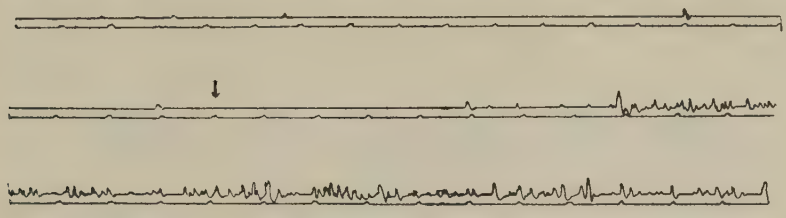

FIG. 1.

determination. The lower line is the time in seconds; the arrow notes the time of the injection.

TABLE I.

No. of Experiment	Position of detector. Costal space	Circ. time Sec.	R. Act. Dep. M. C. injected	Diagnosis
118	4 left	4.5	1.5	Normal
119	4 left	10.0	3.6	Normal
121	4 left	12.0	3.1	Normal
122	4 left	9.0	4.3	Normal
123	4 left	4.5		Normal
126	4 left	9.5	4.4	Cerebr. Hemor.
132	2 right	8.5	3.4	Art. Scler.
133	2 right	10.5	4.2	Normal
134	3 right	9.0	3.4	Normal
135	3 left	12.0	4.0	
140	3 sternum	13.0	3.1	Normal
127	4 left	10.0		Aort. Insuff.
150	3 sternum	8.0	2.5	Normal

Table I contains the data of thirteen venous velocity measurements in thirteen individuals.

Table II shows the results of duplicate determinations in nine individuals.

The trustworthiness of the method is verified by the close correspondence of the results. The one wide variation in the results occurred in determinations 139 and 158, where the former was obtained while the patient showed auricular fibrillation; the latter, after the restoration of normal rhythm by quinidine sulphate.

TABLE II.

No. of Determinations A	Position of Detector Costal Space	Venous Circ. Time Sec. A	R. Act. Dep. M. C. Injected	No. of Determinations B	Position of Detector Costal Space	Venous Circ. Time Sec. B	R. Act. Dep. M. C. Injected	Diagnosis
125	4 Left	12.0	4.1	128	4 Left	10.5	3.1	Cerebr. Hemor.
129	4 Left	9.5	4.8	124	4 Left	5.5	5.2	Normal
130	2 Right	7.0	3.1	131	2 Right	7.5	2.3	Normal
136	3 Sternum	14.0	3.4	146	5 Left	12.0	4.5	Art. Scl.
147	3 Sternum	7.5	3.5	153	3 Sternum	6.5	3.2	Rheum. Fever
149	3 Sternum	9.5	3.5	154	3 Sternum	10.0	2.4	Rheum. Fever
152	3 Sternum	19.0	3.6	155	3 Sternum	15.5	3.1	Malignancy Deep jaundice
151	3 Sternum	4.5	3.4	156	3 Sternum	6.0	3.2	Thromboangitis
139	3 Sternum	15.0	3.4	158	3 Sternum	8.0	3.6	Obliterans A. Aur. Fibr. B. Regular

Knowing the time of arrival of the active deposit in the right heart, and knowing the time of the arrival of the active deposit in the large arteries near the heart, we possess a definite means of gauging the pulmonary circulation time in man.

3123

The effects of loss of skin and of muscle on the development of spinal ganglia.

S. R. DETWILER.

[*From the Zoological Laboratory, Harvard University, Boston, Mass.*]

In previous experiments on embryonic limb transplantation in amblystoma,[1] it was shown that when the fore limb is removed, the sensory ganglia of the brachial nerves (third, fourth and fifth) undergo a decrease in the number of cells of approximately

[1] Detwiler, S. R., *Proc. Nat. Acad.*, 1920, vi, 96.

fifty per cent. Conversely, when a limb is grafted to a strange region, the ganglia of the spinal nerves supplying the heterotopic limb undergo an increased cellular development of twenty-five to forty per cent.

Since it is known that spinal ganglia contain the cell bodies of sensory fibers to skin, as well as to body musculature, and since cellular hypoplasias result when the peripheral field is diminished, the supposition is that both skin loss and muscle loss play a rôle in the resulting hypoplasias.

In an attempt to determine quantitatively the part played by skin and by muscle in the development of spinal ganglia, two embryos were fused together laterally, and in such a way as to reduce greatly the area of skin on the fused sides of the two component embryos. By comparing the area of skin belonging to the fused sides of the components with that covering the free sides, the percentage reduction of skin resulting from the fusion has been obtained for homologous regions of two pairs of "parabiotic twins."[2] By comparing the weight of the musculature belonging to the fused body walls of the two components with that of the two free body walls, the percentage reduction in the volume of muscle was likewise obtained. The cellular reductions in the spinal ganglia, which supply the fused body walls, were also calculated for those nerves concerned with the homologous regions under comparison.

Designating the fraction of cells in the ganglia related to skin by *a*, and the fraction related to muscle by *b*, the data obtained from the quantitative study of skin loss, muscle loss, and cellular loss in the homologous regions of the laterally fused animals, have been utilized to obtain values for *a* and *b*, which indicate that approximately sixty per cent of the cellular losses in the spinal ganglia, after fusion, is due to skin loss, and that approximately forty per cent is due to muscle loss. These values are based upon limited data and further experiments will be made to test their correctness.*

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[2] Burns, R. K., *J. Exp. Zool.*, 1925, xlii, 31.

* A more complete account of the work will appear in the *J. Exp. Zool.*

3124

Epinephrin reaction in obesity.

C. I. KRANTZ and J. H. MEANS.

[*From the Metabolism Laboratory, Massachusetts General Hospital, Boston, Massachusetts.*]

The reaction to epinephrin in the obese has been studied from a metabolic standpoint in a series of cases and compared with a group of normal controls. The subjects were studied in the basal condition, that is twelve to fifteen hours after the last meal, with a period of preliminary rest of one half hour before readings were begun. A solution of adrenalin (P. D. & Co.) in the tablet form, containing 0.625 mg. of adrenalin, were injected intramuscularly in each subject and the metabolism, respiratory quotient, ventilation, pulse rate and blood pressure noted 10, 20, 30, 60, 90, 120, and 150 minutes after epinephrin injection. The tablet form of epinephrin was used so as to insure greater constancy in epinephrin content, the solution having a tendency to deteriorate on standing.

Expressed on a basis of percentage rise above basal, it was found that the metabolism rose 26 to 28 per cent above basal in 20 to 30 minutes after epinephrin injection, in both the obese and normal subjects, which compares favorably with Boothby and Sandiford's work.[1] The ventilation per hour rose to 38 per cent in the obese and to 42 per cent in the normal controls. The respiratory quotients however showed the greatest variation, rising to 11 per cent above basal in 10 minutes in the obese, while a level of 19.6 per cent was reached in the normal subjects in the same length of time. The average basal respiratory quotient in the obese was .754, while in the normal it averaged .810. The pulse rate rose to 40 per cent in the obese, but only to 26 per cent in the normal in 30 minutes after injection. Here the discrepancy is probably due to the lower basal level in the obese, being 65 beats per minute in these cases, while 70 was the average basal level in the normal controls. Pulse pressure reached a height of 57 per cent above basal in 30 minutes in the obese subjects and 67 per cent in the normals in the same length of time.

[1] Boothby, W. M., and Sandiford, Irene, *Am. J. Phys.*, 1920, li, 200.

It will be seen that the rise in metabolism and in ventilation after epinephrin injection in the obese subjects showed no significant difference from that in the normal controls. There was a lower basal respiratory quotient in the obese, and a smaller rise after epinephrin in the obese subjects than in the normal controls. Pulse pressure also showed a slight decrease in rise in the obese when compared to the normal.

3125

The effect of thyroid on calcium metabolism.

CLARK W. HEATH, WALTER BAUER and JOSEPH C. AUB.

[*From the Laboratories of the Massachusetts General Hospital, Boston, Mass.*]

Patients with exophthalmic goiter and normal controls were given a carefully weighed diet deficient in calcium (0.1 gm. per day) but adequate in total calories. On this regime a negative calcium balance was established in all subjects. Determinations were made of total calcium, nitrogen and phosphorus in urine and feces. Frequent basal metabolic rates and determinations of calcium and phosphorus of blood were also made.

Three typical, rather severe cases of exophthalmic goiter showed a very high calcium excretion—one of them five times the average found in a series of controls. Phosphorus excretion was also increased though not as markedly as calcium. This high excretion was maintained with a high basal metabolic rate, but as the basal metabolic rate fell (following the ingestion of Lugol's solution and operation), the calcium excretion also fell markedly and approached normal.

One myxedema patient showed a calcium excretion below normal. Two of the normal controls also took thyroid and thyroxin in amounts sufficient to raise metabolism twenty per cent. On this diet, inadequate in calcium, the calcium excretion rose definitely with the metabolic rate.

In all of these subjects the blood calcium and phosphorus was normal.

The significance of these findings and their possible relation to parathyroid activity is being further investigated on cases of myxedema, adenoma of the thyroid, parathyroid tetany, and on animals.

3126

The mechanism of the postural contraction (tonus) of skeletal muscle.

JOHN F. FULTON. (Introduced by Walter B. Cannon).

[*From the Laboratory of Physiology, Oxford University, England, and from the Laboratories of Physiology, Harvard Medical School, Boston, Mass.*]

Those who find inadequate the belief that the sympathetic nervous system controls the tonus of skeletal muscle have been confronted by the difficulty of offering an alternative view satisfactory to themselves and to their opponents. It is the purpose of this communication to suggest a mechanism of tonic contraction compatible with the all-or-none principle of muscular activity. The present conception takes due account of the long-sustained character of postural reactions; it recognizes the lengthening and shortening reactions, and it conforms with the well-recognized cooperative interaction between voluntary and tonic responses. Finally, it takes into account the probable function subserved by the sympathetic nerve supply of skeletal muscle

To examine tonic reactions of skeletal muscle, it is essential to simplify experimental conditions to the greatest possible extent. Consequently, if we utilize the muscles of a decerebrate preparation, which are admittedly tonic, we must exclude all possible extraneous reflex influence. Magnus[1] has found that decerebrate rigidity continues to exist in preparation, the brain-stem of which has been sectioned just above the vestibular nuclei and in

[1] Magnus, R., "Körperstellung," Berlin, Springer, 1924.

which the VIIIth cranial and the I, II, and III cervical nerves on both sides have been severed. The rigidity of a decerebrate preparation continues to exist after complete denervation of the skin in any given limb, and in any given extensor muscle, after section of all nerves except the nerve supplying that muscle. If one examines the vastocrureus muscle of a decerebrate preparation, the skin and all other muscles of both hind extremities of which have been denervated, one finds that so long as the muscle is stretched the rigidity continues to exist. Detach the tendon and allow the muscle to shorten a few millimeters, and the "rigidity" vanishes. Extend the muscle, and postural contraction once more appears, as may be demonstrated readily by application of an ipsilateral inhibitory stimulus to any afferent nerve of the limb for the muscle then reflexes. This response to extension is the stretch reflex, and it is synonymous with muscle tonus. It disappears forever, once appropriate posterior or the anterior roots have been severed.

When the stretch reflex is elicited by a gradual and uniform extending force, it may be impossible, or practically so, to record any action currents from the responding muscle, even though the *active* tension developed may be of the order of several kilos (as shown by subsequent inhibition). When, however, the extending force is applied in abrupt increments, an action current is observable in the muscle after an interval of $\pm 7\ \sigma$ following each irregularity in the stretch stimulus. Since $7\ \sigma$ is the latency of the knee-jerk, it follows that the observed action current is due to the synchronous character of the stimulus. When elicited by a gradual stretch, on the other hand, the proprioceptive afferent organs are stimulated in sequence, and the resulting temporal dispersion of activity among the constituent reflex arcs renders it impossible to observe action currents in the muscle as a whole. This gives strong *a priori* ground for the belief that the tension of a stretch reflex is maintained by the all-or-none mechanism. Proof that this is the case is provided by the following observation. (Fulton and Liddell.)

When, after a stretch reflex has been elicited, a strong inhibitory stimulus is applied to an appropriate afferent nerve, the *rate* of the ensuing relaxation approximates the rate of relaxation of a motor nerve tetanus. The initial portion of the stretch-reflex re-

laxation is, however, convex upwards for a short distance, which gives evidence of temporal dispersion in the cessation of fibre activity, thus harmonizing with the observations on the electrical responses. If the tension were maintained by a special "fixing" mechanism it would be inconceivable that it could be caused to relax at the same rate as a motor nerve tetanus.

In 1912 Barbour and Stiles[2] suggested, in referring to certain forms of reflex tetanic response, that "this form of nervous discharge must give rise to alternate movements in neighbouring elements of the muscle, one set of fibres shortening while others relax. The resulting tension may, however, be fairly uniform." Evidence has been given elsewhere to show that a similar form of rotational activity occurs in the stretch reflex, thus accounting for its relative unfatiguability. A stretch reflex may be maintained for hours at a time. Since stretch is the adequate stimulus for the maintenance of this form of response, shifting of the incidence of stretch among the various responding afferent end-organs has been suggested as the probable cause of the rotational activity.

Recent evidence,[3] moreover, has indicated that the stretch reflex is the shortening reaction. At whatever length a tonic muscle may happen to be (within certain limits), extension, if not too severe, causes reflex contraction. The lengthening reaction, on the other hand, is a reflex inhibition resulting from an extension so severe as to stimulate the proprioceptive inhibitory endings.

Voluntary contraction dovetails into tonic contraction, and all available evidence indicates that voluntary activity is interpretable on the basis of the same all-or-none mechanism. There is some justification for Hoffman's[4] view that voluntary contraction is merely myotatic contraction caused by the liberation of the lower spinal centres from cortical inhibition.

The painstaking investigations of Boeke,[5] Dusser de Barenne,[6]

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[2] Barbour, G. F., and Stiles, P. G., *Am. Physiol., Ed. Rev.*, 1912, xvii, 75.

[3] *Cf.*, Ch. xvii of the author's "Muscular contraction and the reflex control in movement." (In press.)

[4] Hoffmann, P., "Sehnenreflexe," Berlin, Springer, 1922.

[5] Boeke, J., "Libro en honor de D. S. Ramon y Cajal," Madrid, 1922, p. 113.

[6] Boeke, J., and Dusser de Barenne, J. G., *Proc. Konin. Akad. Wet.*, 1919, xxi, 927.

Kuntz and Kerper,[7] and others,[8] have provided unimpeachable proof of the existence of sympathetic nerves supplying skeletal muscle fibres. Though the evidence now at hand excludes the interesting possibility that these sympathetic fibres are concerned in the maintenance of tonus, it is nevertheless evident that they must subserve some function, and it is the duty of physiologists to recognize them and to discover what they do. The first positive evidence in this direction has been supplied by L. A. Orbeli[9] of Leningrad. He has demonstrated that stimulation of the sympathetic nerves supplying a skeletal muscle (free from its circulation), accelerates recovery if the muscle is fatigued. In this respect activity of the sympathetic has the same action as adrenalin. Increased susceptibility to fatigue following sympathectomy is the one point upon which all observers who have performed this operation agree. This accordingly lends weight to Orbeli's contention that the sympathetic nervous system increases the "efficiency" of muscular activity. Stimulation of the sympathetic would indeed appear to facilitate the removal of acid metabolites. However, we need to know more about the effect of the sympathetic upon the lactic acid mechanism and upon respiratory exchange before we can define the function of the muscle sympathetics with accuracy.

Full discussion of the questions raised in this communication is given in a forthcoming monograph on "Muscular contraction and the reflex control of movement."

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[7] Kuntz, A., and Kerper, A. H., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 25.

[8] Agduhr, E. L., *Proc. Konin. Akad. Wet.*, 1919, xxi, 930 and 1231.

[9] Orbeli, L. A., Pavlov Jubilee Volume, Petrograd, 1924, 429. See also *Brit. Med. J.*, 1924, ii, 633.

3127

Observations on Gye's work with the Rous sarcoma.

J. HOWARD MUELLER.

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The writer wishes to report briefly the results of experiments carried out during the last seven months in attempting to repeat and amplify the experiments of Gye[1] on the Rous chicken sarcoma. The latter interpreted his results as meaning that two factors were involved in the production of this tumor; first a specific, non-living, chemical factor found only in extracts of the neoplasm; and second, a filtering virus, capable of being cultivated *in vitro* under certain conditions. The latter was also obtainable from cultures of mammalian tumors. Neither substance alone was capable of producing a tumor, together they were active. The media used in cultivation of the virus contained broth, rabbit serum and chick embryonic tissue. No controls using uninoculated, but incubated media of this type to replace virus were quoted in the original paper. In personal communications, Gye has reported to the writer that such controls have been carried out and in his hands have proved negative. Murphy,[2] on the contrary, has recently presented evidence that media uninoculated with tumor, but containing either chick embryonic tissue or mouse placenta, i. e., rapidly growing tissues, and incubated anaerobically, could be substituted successfully for virus cultures. Until agreement can be reached on this and other points, judgment of the interpretations to be placed on the phenomenon may well be reserved.

In this laboratory the repetition of the type experiments of Gye have proved to be far from easy. The experiments thus far have used something over two hundred chickens and have served to point out the difficulties to be expected, and perhaps to suggest means of overcoming them.

There are marked individual variations in susceptibility to

[1] Gye, W. E., *Lancet*, 1925, ccix, 109.

[2] Murphy, James B., *J. Am. Med. Assn.*, 1926, lxxxvi, 1270.

small doses of tumor extract manifested even by pure Barred Rock chickens, which we have used exclusively. This may apparently be overcome to a large extent by the use of fairly young fowls of approximately the same age. We are at present using chicks of six to eight weeks of age, hatched from eggs coming as far as possible from the same flock of hens, incubated and raised in the laboratory. Much importance may rest on whether tests and controls are carried out on the same or different chickens. Criticisms of either method may be advanced.

Most important of all the technical points is undoubtedly the preparation of the specific factor by the destruction of virus in the tumor filtrates by means of chloroform. (The terminology of Gye's conclusions is here used for the sake of simplicity.) This was emphasized by Gye in his publication, but an additional difficulty is introduced by the fact, not mentioned by him, and perhaps not true of the tumor in his hands, that the susceptibility to chloroform of the virus obtained from tumors in different chickens appears to vary tremendously. In the experiments summarized here, roughly half of the filtrates treated with chloroform have failed to produce tumors either alone or when mixed with virus cultures. In the other half, tumors have been produced by both the mixture and the control. In other words, about half of the chloroform filtrates have been too severely inactivated by the reagent, and in the case of the others the treatment has been too mild. Obviously, no standardization involving quantity of chloroform, time of treatment and method of mixing will be of any avail as long as individual tumors show marked differences in their response. At present the writer is attempting to overcome this difficulty by departing rather widely from Gye's actual technique by the use of dessicated tumor in place of fresh tumor in the preparation of filtrates. As Rous, Murphy and Tytler[3] showed in their early work on this tumor, drying does not destroy the infectious agent. By preparing a sufficient quantity of dessicated tumor to carry out a number of experiments it should be possible to so standardize the method as to give uniform filtrates which can be brought with some certainty by means of chloroform to a point where it will just fail to infect most chickens in given

[3] Rous, P., Murphy, J. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lviii, 1682.

dosage. Results up to the present are encouraging. Filtrates prepared from dessicated material are infectious in approximately the same quantities as those prepared from corresponding amounts of fresh tumor tissue. They are apparently rendered inactive rather more easily by chloroform than the latter. It is too early yet to say how uniform the resistance to chloroform will prove to be. Moreover, in two chickens out of four in one group injected in one breast with a mixture of a chloroform filtrate and a virus, and in the other with the chloroform filtrate alone, tumors have developed from the latter injection, and not from the former.

It seems that the greatest hope of clearing up the question involved in this work must come through a method which will give predictable results in controls and type tests in all, or nearly all chickens inoculated. We have had a few experiments, not more than two or three, in which it would be possible to pick out from a dozen chickens inoculated three or four in which a perfect type experiment was shown. In one experiment in particular, owing to a peculiar and entirely accidental grouping of controls and tests both on the same and on different chickens, it is not unlikely that positive results were really obtained, although certain of the controls were also positive. There is apparently reason to believe that by means of a uniform dessicated preparation, similar results may be obtained in the majority of experiments, and it will then be possible through an extension of controls to arrive at a more exact understanding of the reasons underlying Gye's experiments.

To summarize, in order to repeat and more adequately control Gye's experiments, it is essential to standardize every variable factor as far as possible. We believe the differing susceptibility on the part of the chickens may be largely overcome by using young chickens of about the same age from the same blood related flock. The most important variable is the resistance to chloroform of individual tumors. It appears that this may be largely obviated by substituting dessicated material made in considerable quantity for fresh tumor in the preparation of the specific factor. While we have obtained occasional indications of successful experiments of Gye's type, little can be learned from them unless they can be produced with considerable uniformity.

Many of these experiments have been carried out with the assistance of Miss Alberta Marx, and Mr. Ashton Graybiel, to whom I wish to express my appreciation and thanks.

3128

Extent of capillary bed and rôle of Thebesian vessels in coronary circulation.

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Intracardiac injections of dyes and of India ink in living cats, rabbits and rats have resulted in complete filling of the capillaries of these hearts. Numerous counts have shown approximately 1100 capillaries to each thousand muscle fibers, or about one capillary to each muscle fiber. But when for any reason the heart dilated during the injection, very few of the capillaries were injected, though the larger vessels were completely injected.

The same results were obtained when human hearts were injected through the coronary arteries. Distension of the chambers prevented injection of the capillaries, but when steps were taken to prevent dilatation of the chambers complete injections were obtained.

At the same time it was noted that perfusion of the coronary arteries in dead hearts resulted in distension of the chambers, and when the walls were so stretched 80 per cent to 90 per cent of the perfusate escaped directly into the chambers of the heart, while only 10 per cent to 20 per cent returned by way of the coronary sinus and veins. These findings suggest that during dilatation of the heart the chief route of blood flow is through the arteries to the Thebesian vessels and thence into the chambers of the heart.

A modification of the Langendorff method of coronary perfusion has given complete injections of the capillaries in cat and rabbit hearts when the hearts were beating strongly. This meth-

od when applied to human hearts obtained within three or four hours post mortem has given complete injection of the capillaries in certain areas of the heart. There is approximately one capillary per muscle fiber in the human heart, except in the auricle where the number is not constant and the supply less abundant.

By the methods described above it has also been possible to obtain good injections of the vessels of the heart valves and of the capillaries in the wall of the aorta. In one instance the vessels in a papillary muscle were seen to anastamose with those coming down from the base of the valve cusp.

Further studies upon the quantitative distribution of capillaries in the heart and upon the function of the Thebesian vessels are now in progress.

3129

Heterakis vesicularis Frölich 1791: A vector of an infectious disease.

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Several parasites, a flagellate of the genus *Giardia* and also microsporidia have been reported in intestinal round worms, but up to the present time the latter have not been shown to be concerned in the transmission of disease.

Blackhead, an infectious disease of turkeys and other poultry, is caused by a flagellate, *Histomonas meleagridis*. It is transmitted experimentally and to some extent in nature by the direct ingestion of material contaminated with freshly passed discharges containing the protozoon. It appears, however, to be much more frequently transmitted indirectly by some phase distributed on the soil, evidently in association with the eggs of the caecal worm, *H. vesicularis*.

The presence of the protozoon in the egg of *Heterakis* is indicated by experimental evidence of various sorts.

1. *Heterakis* eggs kept in 1.5 per cent nitric acid until embryonated produce blackhead when fed to young birds isolated from all other sources of infection, although this treatment renders the material bacteriologically sterile.

2. That there occurs no resistant phase of the blackhead protozoon apart from the worm egg, capable of resisting the 1.5 per cent acid, is shown by the invariably negative results obtained by the repeated feeding of susceptible birds with the discharges of blackhead carriers, after treatment in 1.5 per cent acid. Furthermore, no resistant form has been demonstrated microscopically.

3. *Heterakis* material will only produce blackhead after the ova have become embryonated and capable of hatching. Samples of the same material fed before the eggs are ripe invariably furnish negative results.

4. The feeding of male *Heterakis* also furnishes only negative results although ova-containing females of the same lot produce blackhead.

The disease usually follows the feeding of large numbers of *Heterakis,* especially when the latter are pooled from several different birds. However, it is occasionally possible to feed large numbers from a single bird without producing blackhead. *H. vesicularis* obtained from pheasants has also furnished only negative results, as well as that obtained from the goose.

Morphological evidence of the presence of the blackhead flagellate in the ovum of *Heterakis* has not yet been obtained, although large numbers of eggs have been examined. However, the invasion of the tissue of the worm by the protozoon has been demonstrated in a number of instances, thus far in half-grown worms from cases of blackhead. It is not yet known whether the acute disease or the carrier state is the most favorable for the infection of the worm.

3130

Observations on the diastase activity of the blood of infants.

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In 1917 Myers and Killian[1] described a new simple method for the measurement of the diastatic, or starch-splitting, activity of blood. In this method two samples of 2 cc. each of oxalated blood, diluted with a given amount of water, to one of which was added 1 cc. of a 1 per cent solution of soluble starch, were heated in a water bath for 15 minutes at 40° C. and at the end of the digestion period the sugar content of each sample was measured. The difference, representing the amount of glucose liberated from the starch by the blood diastase, was converted into per cent of the original amount of starch (10 mg.), and this percentage figure was spoken of as the diastatic index or as diastatic units. They reported a considerable number of findings from the examination of the blood of healthy and diseased individuals, and later DeNiord and Schreiner,[2] Brill,[3] Watanabe,[4] and Lewis and Mason[5] reported clinical studies in which this method was used with slight modification. None of the results reported, however, gave evidence of constant variations in this diastatic power of the blood, which could be definitely correlated with the physical condition of the individuals studied. Karsner, Koechert, Wahl,[6] and Cohen[7] have reported studies of the variation of the blood diastase in animals under certain experimental conditions, using this method.

A study of the diastatic activity of the blood of infants and children, normal and otherwise, was undertaken with the hypo-

[1] Myers, V. C., and Killian, J. A., *J. Biol. Chem.*, 1917, xxix, 179.

[2] De Niord, H. H., and Schreiner, B. F., *Arch. Int. Med.*, 1919, xxiii, 484.

[3] Brill, I. C., *Arch. Int. Med.*, 1924, xxxiv, 542.

[4] Watanabe, C. K., *Am. J. Physiol.*, 1917, xlv, 30.

[5] Lewis, D. C., and Mason, E. H., *Am. J. Physiol.*, 1920, xliv, 455.

[6] Karsner, H. T., Koeckert, H. L., and Wahl, S. A., *J. Exp. Med.*, 1921, xxxiv, 349.

[7] Cohen, S. J., *Am. J. Physiol.*, 1924, lxix, 125.

thesis that the blood of infants suffering with various types of severe nutritional disturbances might show significant variations in the activity of the enzymes presumably responsible, in part, for the utilization of food substances. A brief summary of the findings among the infants will be presented.

In these studies the method of Myers and Killian has been changed somewhat for reasons which will not be given here, the principal change being the adoption of a digestion period of 1 hour instead of 15 minutes. Ordinary commercial corn starch was used instead of soluble starch because the samples of the latter which were available contained reducing sugars; the corn starch was free from such reducing substances and gave constant results in repeated check experiments. The dry incubator temperature of 37° C. was chosen for convenience, although the diastatics ferment is slightly more active at 40°.

Method: 2 cc. samples of oxalated blood placed in two 50 cc. Erlenmeyer flasks. One is diluted with 14 cc. H_2O, the other with 13 cc. H_2O, and the two placed in an incubator at 37° C. for one-half hour, to the second is then added 1 cc. of a 1 per cent starch solution and the two left at 37° for 1 hour. At the end of this digestion period the sugar content of each is measured by the Folin method,[8] they being ready for precipitation with the Na tungstate and H_2SO_4 solutions without further dilution. The difference between the values so obtained (the values being expressed as mg. of glucose per 100 cc. of blood) has been taken as an index of the diastatic activity of the blood sample; this figure actually represents the number of mg. of glucose which would be liberated in a proportionate mixture of 100 cc. of blood and 50 cc. of 1 per cent starch solution, under similar conditions.

In the normal infants and children studied there is a definite increase in the diastatic activity of the blood according to age; for infants of 2 months the index as here expressed is usually 20, while 35 to 40 is the average figure for those approaching 2 years, and for older children the average normal value is slightly higher.

The table given summarizes the findings from examinations of blood samples from approximately 100 infants less than 2 years of age.

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[8] Folin, O., *J. Biol. Chem.*, 1926, lxvii, 357.

	Diastatic Index	
	Extremes	*Average*
1. Normal infants, and those admitted to the hospital for "Regulation of feeding"—cases apparently free from complicating infections. *16 cases:*	18–35	26
5 determinations among these cases, while they were doing poorly:	2–21	11
10 determinations among these cases, while they were doing well and gaining weight:	18–49	30
2. "Nutritional secondary anemias" (included the Von Jaksch type): *6 cases:*	20–58	44
3. Upper respiratory infections and pneumonia. *16 cases:*	25–146	58
4. Miscellaneous group of cases of generalized tuberculosis, congenital syphilis, rickets, meningococcus meningitis. *11 cases:*	20–51	33
5. "Acute intestinal intoxication" of varying degrees of severity. *43 cases:*	0–25 (1 case, only, above 18)	10
6 determinations among these cases shortly *preceding* the onset of the acute illness:	15–58	26
18 determinations among these cases *after* recovery:	20–54	33
6. Recovered cases of "Intestinal intoxication" (infants admitted to the hospital shortly after their illness, for other reasons; they were all apparently doing well) *5 cases:*	22–36	28

From the above data and experimental studies which have paralleled them, no definite conclusion has been drawn as to the significance of the striking changes in the starch-splitting activity of the blood of these infants, but it seems that the described index of this activity is fairly constant in those children in a good nutritional state, gaining weight and presenting no dietary difficulties, and that it is almost invariably diminished during certain of their acute nutritional disturbances. Most of the infants

showing the marked diminution in this index had diarrhea, either at the time of, or just previous to, the blood examinations, and since it is so generally believed that the amylolytic ferment of the blood is derived from the digestive juices, its diminution might be explained as due simply to exhaustion of the supply of the ferment by the profuse bowel movements. However, in the cases classified as intestinal intoxication, most of them being the familiar infantile summer diarrhea, the lowering of the diastatic index was not proportional to the severity or duration of the diarrhea but rather to the severity of the condition described clinically as "intoxication and dehydration"; moreover, some of the infants suffering more chronic nutritional disturbances, without diarrhea, during their most unfavorable periods have shown blood diastase values as low as those observed in cases of acute diarrhea. This observation seems to justify the conclusion that the diarrhea *per se* is not the important factor in diminishing the diastatic index. Whether during these disturbances a suppression of the secretion of the intestinal juices, with parallel lowering of the concentration of the enzymes in the blood, is an important factor in the infant's intolerance of food is a question open to speculation. The onset of pneumonia in infants is usually heralded by profuse diarrhea, yet the blood of these infants with pneumonia has shown markedly increased diastatic activity.

Experimental studies have shed little light on the significance of these observed changes. In rabbits certain bacterial toxins, particularly diphtheria toxin, have caused great increases in the blood diastase activity, changes comparable to those increases found in pneumonia in the infants, but as yet there has been found no way of causing noteworthy diminution of this activity in experimental animals.

Western New York Branch

University of Rochester Medical School, April 17, 1926.

3131

A sensitive method for measuring carbon dioxide.

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The most sensitive method hitherto used for measuring the carbon dioxide output of tissues *in vitro* is the method of Tashiro,[1] depending upon the detection of a crystal of barium carbonate in a solution of barium hydroxide under the microscope. It was found that 1×10^{-7} of a gram of carbon dioxide gave a visible precipitate in 10 minutes. An unknown amount of carbon dioxide was measured by finding such a dilution that a visible precipitate was just formed in 10 minutes. This method has not been found useful in practice in the hands of others.

The next most sensitive method is the indicator method of Osterhout,[2] which in the hands of Parker[3] has been found adequate for the detection of 1×10^{-6} grams. It takes, however, 20×10^{-6} grams to produce the standard color change in the indicator. If this change takes place in 20 minutes, Parker finds himself able to detect a perceptible color change after 1 minute, *i. e.*, from the effect of 1×10^{-6} grams.

At the Cleveland meeting of the Federation, December, 1925, the writer demonstrated a carbon dioxide method based upon measurements of the conductivity of barium hydroxide, with which it is possible to detect 1×10^{-7} of a gram of carbon diox-

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[1] Tashiro, S., *Am. J. Physiol.*, 1913, xxxii, 107.

[2] Osterhout, W. J. V., *J. Gen. Physiol.*, 1918, i, 17.

[3] Parker, G. H., *J. Gen. Physiol.*, 1925, vii, 641.

ide. The method was designed for the estimation of the carbon dioxide output from a stimulated nerve. Owing to an unavoidable delay in completing the experiments on nerves it has seemed advisable, in response to requests from other workers, to publish a brief description of the method, which is not new in principle. It was pointed out to me at Cleveland that it had been used by Spoehr[4] for relatively large amounts of CO_2.

Conductivity measurements are made in 7 cc. of a solution of barium hydroxide 0.00475 M. This solution is contained in a tube 10 cm. long and 12 mm. outside diameter, supplied with electrodes 5.7 cm. apart. Air is introduced into this tube from the respiration chamber and passes through the solution in a row of bubbles. The bubbles emerge from the surface of the solution into a bubbling chamber and return through two valves to the respiration chamber again. Circulation of the air is accomplished by a rising and falling mercury surface placed between the two valves. The valves are made of tapering glass tubes about 18 mm. long and 4 mm. in diameter, filled with mercury, sealed off and carefully ground into their seats until air tight. The mercury surface is made to rise and fall by a levelling bulb actuated by an automobile windshield cleaner. The mercury surface is covered with oil to avoid mercury vapor in the apparatus. It is important to keep the rate of circulation of air constant throughout an experiment. This rate is recorded by the addition of a side arm on the respiratory chamber over which is fitted a small rubber balloon. The balloon is enclosed in a glass bulb, one outlet from which goes to a volume recorder, which writes on a drum. The excursion of the volume recorder follows faithfully the movements of the mercury surface. Carbon dioxide is carefully excluded from the air space between the balloon and the volume recorder. The respiration chamber may be of any convenient shape, preferably as small as possible. In my apparatus it is about the size of an ordinary 6 inch test tube, closed at the top by a ground glass stopper and communicating with the absorption chamber by a narrow tube at its base. The whole circuit is, therefore, of glass with the exception of the balloon, which may be omitted except when needed as a pressure equalizer in special experiments. Stop cocks are sealed into the circuit on either side of the valves to assist in regulating the air

[4] Spoehr, H. A., *J. Ind. and Eng. Chem.*, 1924, xvi, 128.

content of the apparatus, introducing particular gases according to needs and in drying the valves (by a current of air) in case they become moist. The temperature of the water bath is regulated to ±0.001° C. The conductivity is measured by a Leeds Northrup 470 cm. slide wire, a resistance box of Curtis coils, using an audio oscillator as a source of current. With this arrangement the accuracy depends upon the ability to maintain a constant base line rather than upon the accuracy with which the conductivity is measured. Aside from the rigid exclusion of outside air from the apparatus and general cleanliness, the regularity of the base line depends largely upon smooth working of the valves and the regularity of pumping. The conductivity change is never quite zero, even in the absence of tissue in the respiration chamber, and a small correction must be applied for absolute values. This may depend upon a reaction between the solution and the glass. A similar objection applies to the colorimetric method.

3132

On the phase reversal of the lipoid-aqueous systems in the bacterial cell wall.

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In previous communications, Mellon[1, 2] has shown that the principle of ion antagonism is applicable to bacteria. Under the conditions of the experiment with strain N.D.—67 the principle operated in a Na:Ca ratio as high as 100 or 150 to 1. This strain, which is quite stable in H_2O and NaCl solutions, is quickly precipitated by $CaCl_2$ solutions in strengths as dilute as 10^{-6} cm. The physico-chemical mechanism suggested was reversal of the aqueous-lipoid system in the wall of the cell whereby $CaCl_2$

[1] Mellon, Ralph R., *J. Med. Res.*, 1922, lxiii, 345.

[2] Mellon, Ralph R., Hastings, W. S., and Anastasia, C., *J. Immunol.*, 1924, ix, 365.

made the lipoid layer the external phase. This theory is in accord with the work of Clowes[3] with the simple oil-water emulsions.

It was determined to test the validity of this conception by the surface tension method. Mudd[4] has shown that the tubercle bacillus when placed at the interface between an aqueous and a lipoid or a lipoid solvent will always be drawn into the latter by virtue of the large amount of wax contained in this organism. It was thought that the occurrence of the same phenomenon would be strong evidence for a reversal of the aqueous-lipoidal relations of our N.D.—67 by $CaCl_2$.

Accordingly the organisms were placed in $CaCl_2$ solution in concentrations ranging from 10^{-1} to 10^{-6} and this solution shaken with organic lipoidal solvents, such as cyclohexanol, amyl alcohol, capryl alcohol and pelargonic acid. In every instance the organisms were found on the organic side of the phase boundary when the latter formed. They did not pass over to the aqueous side under the time of observation, which was several days.

A series of higher alcohols was used that showed a progressively increased solubility in H_2O as follows: Di-butyl carbinol $<$ than Di-propyl carbinol which is $<$ than Di-ethel carbinol. In accordance with the theory, the organisms acted on by $CaCl_2$ did not leave the aqueous phase for the Di-ethyl, but they did so completely for the Di-butyl carbinol, while the Di-propyl was about a 50-50 transference.

Furthermore the organisms when extracted by alcohol:ether 2:1 showed removal of the lipoid, which prevented their affinity for the lipoid solvent. This was shown by the fact that when acted on by $CaCl_2$ they no longer left the aqueous solution for the cyclophexanol. Thus the reversal of phase relations between the lipoid-aqueous systems of the cell wall is supported by these experiments.

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[3] Clowes, G. H. A., *J. Phys. Chem.*, 1916, xx, 407.

[4] Mudd, Stuart, and Mudd, Emily, B. H., *J. Exp. Med.*, 1924, lx, 647.

3133

Lipid excretion by bile fistula dogs on a lipid-free diet.

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Previous work[1] has indicated that, contrary to the generally accepted view, the fecal lipids (particularly the sterols) do not reach the intestine by way of the bile, but enter it below the absorbing portion. In order to shed further light upon this question, bile fistula operations have been performed upon 3 of the normal dogs used in previous work with essentially lipid-free diets[1, 2] and these animals have been employed in similar experiments, the results obtained while the dogs were still normal, serving as controls. In the case of 2 of the animals, 6 such control experiments had been carried out, while 13 were available in the case of the 3rd. No attempt was made to keep the bile tract sterile in one of the animals, and there was considerable infection during the experiment. The dog ate well, however, appeared normal, and maintained its weight. There was no essential difference in the results from this animal and those from the others in which the bile tract was kept sterile. During the experiments the dogs were prevented from licking the bile either by means of a muzzle (in one experiment with the infected dog) or by collection of the bile in a balloon kept in place by a binder. There was no marked loss of weight in any case.

The diet was based on the work of Cowgill[3] and contained casein (23.2 parts), sugar (50.0 parts), bone ash (1.4 parts), Liebig's meat extract (1.8 parts), salt mixture (1 part), and Vitavose* (2.2 parts). The casein was continuously extracted with hot alcohol for several days, and that used in most of the experiments contained less than 2 parts of lipids in 10,000 as determined by digestion of the protein with strong alkali for 24

[1] Sperry, W. M., *J. Biol. Chem.*, 1926, lxviii, 357.

[2] Sperry, W. M., and Bloor, W. R., *J. Biol. Chem.*, 1924, lx, 261.

[3] Cowgill, G. R., *J. Biol. Chem.*, 1923, lvi, 725.

* The author is indebted to the Ward Baking Company for the Vitavose used in these experiments.

hours, followed by acidification and extraction with ether and petroleum ether. The small amounts of lipid material in the meat extract and Vitavose were considered negligible in view of the results obtained.

The analytical procedure consisted briefly, in dividing the feces excreted by the dogs on the above diet, into weekly periods by charcoal demarcation, and separating the lipids obtained after complete saponification into unsaponifiable, non-volatile fatty acid, solid fatty acid, and liquid fatty acid fractions. In all, 12 weekly experiments were carried out, of which 11 formed parts of a series in which the animals were kept on the diet for periods of 3 or 4 weeks. The dogs were fed the experimental diet for 3 or 4 days, and a water enema was given before the marker was fed, in order to eliminate the possibility of stagnation of fat from previous diets in the intestine. An enema was administered on the day following the feeding of the marker and good separation into weekly periods was always obtained.

The most striking feature of the results was a marked increase in the total lipid excretion over that obtained when the animals were normal, from about 2 to 4 times as much material being excreted, without exception, by the animals with the bile fistulas. The amounts varied from 3.076 to 8.663 gm., while the normal average excretion was about 1.7 gm. Continuing the experiments over long periods of time appeared to have no effect. The largest excretion was obtained during the 4th week of one of the series. As in the case of the normal animals, the excretion was quite constant in composition, and the ratio of unsaponifiable material to total fatty acids was almost identical with that found in lipids excreted by normal dogs. The ratio of liquid to solid acids was somewhat smaller in most cases and the solid acids had a higher melting point than those from the normal animals. These differences were not large, however, and in general it may be stated that there is markedly larger excretion of lipids by bile fistula than by normal dogs on essentially lipid-free diets, and that the composition is very nearly the same in both cases.

It has been suggested that these results may be explained by an increase in intestinal bacteria and experiments are being planned which it is hoped will throw light on this point. Perhaps a more plausible explanation is that there is a secretion of lipids throughout the length of the intestine, which in normal animals is largely absorbed but which escapes absorption in bile

fistula animals. At any rate it seems to be strongly indicated that fecal lipids do not necessarily enter the intestine by way of the bile.

3134

A preliminary study of conditioned motor reflexes in thyroidectomized sheep.

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The investigation of habit formation in thyroidectomized sheep and goats by the maze method has yielded results difficult to analyze because of the complicated responses elicited. The conditioned motor reflex method of Bekhterev has, therefore, been adopted.

In a preliminary experiment a conditioned reflex to a tactile stimulus was established in three animals, two thyroidectomized sheep, aged three and four years respectively, and the twin control of the three year cretin. Tactile stimuli were applied to a spot on the rump, at the rate of thirty per minute, for periods varying from two to ten seconds. With the final stimulus, a faradic current was applied to the left foreleg of sufficient intensity to evoke a defensive movement. The first definite leg movement, in response to the tactile stimulus alone, occurred at the tenth combination in the control and in the three year cretin, and at the seventeenth combination in the older cretin. At the end of the ninth day and forty-fifth combination, one milligram of thyroxin was administered to each thyroidectomized animal. In spite of this, one and one half months later the younger cretin died and the training of the other animals was then discontinued, after two hundred thirty-eight combinations of conditioned and unconditioned stimuli.

After an interval of six and one half months the tactile stimulus without reenforcement evoked a vigorous conditioned reflex in both normal and cretin sheep. This is shown in Fig. 1, the

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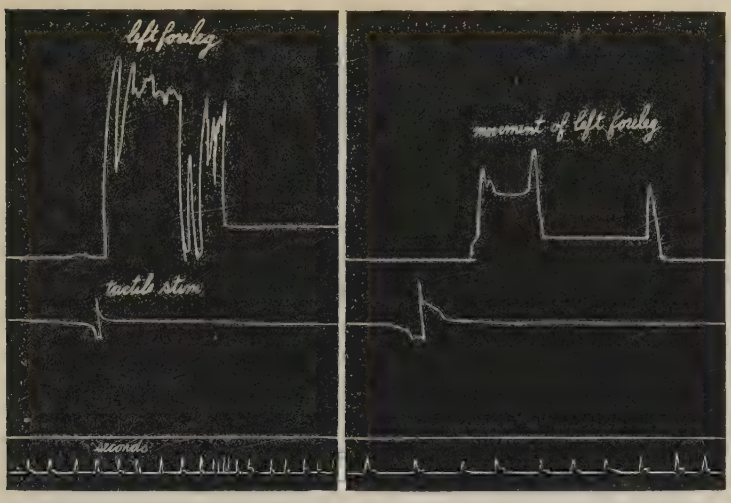

FIG. 1.

tracing from the cretin appearing at the left. Continued tactile stimulation without application of the faradic current soon elicited a less extensive response (see Fig. 2) and finally, after one hundred nine periods of stimulation, distributed over sixteen days, the conditioned reflex failed to appear in either animal on the seventeenth day. This preliminary experiment, therefore, fails to demonstrate any influence of thyroidectomy on the formation, perseveration, or extinction of the conditioned motor

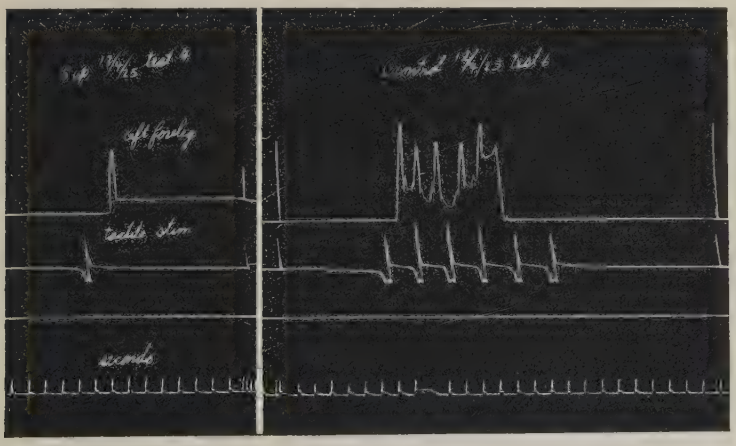

FIG. 2.

reflex of the simultaneous type. It has been shown, however, that during periods of extreme lethargy in the cretin sheep this reflex is weakened.[1]

The suggestion was made[1] that the weakened muscles of the cretin might initiate defective proprioceptive impulses and thus influence behavior in escaping from the maze. The repeated movements of the cretin's foreleg in response to the conditioned tactile stimulus (Fig. 1, at the left) which are believed to be conditioned reflexes to proprioceptive stimuli of secondary origin[2] indicate normal functioning of the proprioceptors in the thyroidectomized sheep.

3135

The effect of diphtheria toxin on the adrenals.*

FRANK A. HARTMAN and J. J. MACDONALD.

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The adrenals are remarkably susceptible to diphtheria toxin. Elliott[1] found that a sufficient dose of diphtheria toxin to produce death within seventy hours after injection usually caused a marked depletion of the epinephrin content of an adrenal with an intact nerve supply.

We have attempted to show an increased output of epinephrin after the injection of diphtheria toxin by means of the deganglionated iris (superior cervical ganglion, removed at least a week before the test). Diphtheria toxin ranging from 0.0005 cc. per kilo to 0.0119 cc. per kilo was injected intraperitoneally into fourteen cats. This was usually given in one injection.

The size of the pupil (as compared to the control), the rectal temperature and heart rate were determined at intervals after the injection.

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[1] Liddell, H. S., *Am. J. Physiol.*, 1926, lxxv, 579.

[2] Beritoff, J. S., *Brain*, 1924, xxvii, 360.

* Aided by a grant from the Elizabeth Thompson Science Fund.

[1] Elliott, T. R., *J. Physiol.*, 1912, xliv, 374.

With the larger doses some animals died in twenty to twenty-six hours. Animals lived longer with the smaller doses, although most of them died from the injection within a few days.

In none of the cats was there an unquestioned dilatation of the deganglionated pupil.

The adrenals were fixed with formaldehyde and potassium bichromate and stained with Sudan III.

With the range of dosage used there appeared to be no definite relation between the amount of toxin and the depletion of the medulla. Occasionally, the medulla was depleted, judging by the staining. Usually, however, epinephrin was present in patches or in limited groups of cells.

Likewise, there appeared to be no definite relation between the dosage and the amount of lipoids in the cortex except that with the larger doses, lipoids were absent or nearly so in the *zona reticularis.*

Pacific Coast Branch

Stanford University, Calif., April 17, 1926.

3136

A simple method of demonstrating muscular hypertonicity in anaphylactic shock: Crop tonus in pigeons.

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[*From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.*]

There has been lacking a suitable, simple method for demonstrating the muscular hypertonicity of anaphylactic shock in the intact organism. The following method answers the purpose admirably.

A sensitized pigeon of about 300 to 400 gm. body weight, and deprived of its food over night, is tied, back down, by fixing its legs and wings conveniently to a small operating board such as is used for guinea pigs. A head holder is unnecessary. Then a balloon made from a fish-skin condom, tightly attached to the end of a rubber catheter (No. 20 French) so as to form a balloon of about 10 by 4 cm. when distended, is introduced directly into the crop. The introduction is facilitated by first collapsing the balloon and then bringing it in close apposition to the catheter, which is stiff and carries the balloon with it. The catheter is now attached to a T-tube, which in turn is joined by rubber tubing to a small tambour. Then the balloon is distended by blowing air through one arm of the T-tube, and the system adjusted so as to keep the crop moderately well distended with air and yet permit a record of tonus changes from the tambour on a slow kymograph. Presently large peristaltic waves may begin, but these are not indispensable to the experiment. As soon as a control record is secured, the antigen is injected into a wing vein (right)

in the axillary region, using an ordinary Luer syringe and needle of 23 or 26 gauge.

Almost immediately after injection of from 0.2 to 0.5 cc. of the antigen, there is a sharp and marked rise in tonus accompanied by other symptoms (lachrymation, salivation, dyspnea, convulsions, etc.) of anaphylactic shock. Since the tonus increase occurs in decapitated, curarized, atropinized, epinephrinized, hepatectomized and anesthetized pigeons, the seat of stimulation is in the crop musculature itself independently of the brain, autonomic nerves, and liver (anaphylatoxin). Moreover, the stimulation is completely removed by papaverine, a direct muscular depressant. Hypodermic, intramuscular and intraabdominal administrations of the antigen are ineffective.

The same method gives interesting and striking effects with physically treated serum and with drugs which will be reported later.

3137

The presence of iron depositing bacteria in milk.

C. S. MUDGE.

[*From the University of California, Davis, Calif.*]

Harder[1] has made a careful study of the group of microorganisms, generally classed by him as the trichobacteria, to which he ascribes the deposition of certain sedimentary iron ores. Although Harder devotes himself largely to these trichobacteria, he mentions finding a number of bacteria (eubacteria) which were also able to precipitate ferric hydroxide and other ferric salts from media containing iron salts of organic acids. Since these iron depositing bacteria are soil types, it is not surprising that to a greater or lesser degree they should be found in milk and its products.

The ferric ammonium citrate media of Harder was used both as a broth and as a solid medium. On the agar were obtained colonies described by Harder as typical; that is, large, irregular,

[1] Harder, E. C., U. S. Geol. Survey Professional Paper 113, 1919.

iron-incrusted colonies. Other types as well were found. Of the organisms forming these colonies, cocci and bacilli seem to occur in about equal numbers. There were very few spore formers, no motility, and no liquefaction of gelatin. Dextrose is fermented but lactose and saccharose only rarely so. As a class these organisms were able to utilize the salts of organic acids for their source of carbon, the pH of the medium rising distinctly. Thus they correspond to the group of alkali bacteria studied by Ayers.[2]

The knowledge of the presence of these organisms in milk is believed to be new and significance is attached to them, since frequently undesirable qualities are found in the milk when they are present in considerable numbers.

3138

The possible rôle of iron depositing bacteria in the formation of hard pan.

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During the studies of iron depositing bacteria as found in milk (see preceding paper) and being impressed with the possible relation of these organisms to hard pan formation, some iron hard pans such as abound in certain sections in the state of California were inoculated into the ferric ammonium citrate medium of Harder.[1] After standing two months a precipitate appeared which is typical of the reaction of iron depositing bacteria in this medium. Plates poured with this medium showed colonies of bacteria (bacilli) and actinomyces. Observation of these pans under the microscope disclosed as well some very minute threads which on staining suggested crenothrix. Not all of the pans, however, showed this appearance.

[2] Ayers, S. Henry, Rupp, Philip, and Johnson, W. T., Jr., U. S. D. A. Bulletin No. 782.

[1] Harder, E. C., U. S. Geol. Survey Professional Paper 113, 1919.

It is recognized that this is hypothetical, but Morrison and Sothers[2] have suggested this very thing, although with no apparent evidence to substantiate their suggestion. And recently A. C. Swinnerton[3] states that bacteria "may be important factors in the cementation of sand and gravel materials."

Work along this line is being continued.

3139

Physical measurements on operated hyperthyroids.

W. R. MILES and H. F. ROOT.

[*From the Psychology Laboratories, Stanford University, California.*]

A better understanding of the relationship of physical measurements to body weight is necessary before a more certain basis for predicting normality of weight can be found. At present height is practically the sole basis. But height is a very stable measure in which adults are probably more nearly alike than for any other physical measurement. Stature has a coefficient of variability of only 4 per cent, weight has around 12 per cent, and pelvic diameter as representative of bony body widths has approximately 9 per cent. If the bony widths of the body have so much variability as compared with bony lengths should they not be taken into account in estimating the normal weight, which of course is a cubic factor?

A group of adult hyperthyroid cases were measured before, and again, six months after operation. Critical linear measures were carefully taken. The effort was for "bony measurement." Data for 14 cases, 11 women and 3 men, are summarized. The average gain in weight was 9.1 kilograms, an increase of 17 per cent of the preoperation weight. The calories per kilogram decreased 37 per cent. Chest girth, a cross-sectional measure, increased 9 per cent, but the bony lengths gave almost no change;

[2] Morrison, C. G. T., and Sothers, D. B., *J. of Agr. Science*, 1914, vi, 84.

[3] Swinnerton, A. C., *Science*, lxiii, 74.

height, + 0.1; sternal notch height, + 0.2, and sitting height, + 0.4 per cent. Widths were not quite as fixed during this marked weight change as were lengths: shoulders (acromion) + 2.3; chest (transverse diameter) + 2.7; chest depth, + 4.2, and pelvic maximum diameter, + 0.5 per cent. The latter measure is remarkable constant with the individual.

Since adults show relatively large differences among themselves in the linear skeletal widths, and, these measures show themselves quite constant in the individual adult, even under marked and rapid weight change, it is evident, if further data confirms, that some skeletal widths should appear in the formula for predicting normal weight.

3140

The crystallization of starch.

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[*From the Food Research Institute and the Department of Chemistry of Stanford University.*]

In the early stages of the malt diastase hydrolysis of starch, Lintner and Düll[1] obtained crystal clusters which gave the characteristic starch reaction with iodine, were insoluble in cold water but soluble in hot, and had a specific rotation of 196°. Beijerinck[2] dissolved starch paste by autoclaving and obtained microscopic needle clusters on cooling. These were birefringent when viewed between crossed nicols, but did not show the black cross which is so characteristic of starch grains. The writers' associate, Van de Sande Bakhuyzen,[3] found that aqueous solutions of starch, prepared by the method of the writers[4] without

[1] Lintner, C. J., and Düll, G., *Ber. d. Deutsch. Chem. Gesellsch.*, 1893, p. 2533.

[2] Beijerinck, M. W., *K. Akad. v. Wetenschapp. te Amsterdam*, Proc. Section of Sciences, xviii, 1, 305.

[3] Van de Sande Bakhuyzen, H. L., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 506.

[4] Alsberg, C. L., and Perry, E. E., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 60.

the use of reagents or heat, give a precipitate with alcohol consisting of clusters of microscopic needles, which when viewed between crossed nicols are birefringent, without showing the black cross.

The writers repeated Beijerinck's experiments and verified his results. However, by autoclaving at a somewhat higher temperature, 150° to 160° C, and by the use of some other modifications of his method, they obtained clusters of larger needles, not merely from potato starch, but also from wheat, maize, canna and arrow-root starches. These crystals are but little soluble in cold water, more readily in hot. They give the iodine reaction.

Viewed between crossed nicols, the clusters are birefringent in all cases. In the case of wheat, maize, and arrow-root, the needles are very small. In the case of the potato and canna, they are relatively large—some of the clusters reaching a diameter over one-half that of the natural granules. The potato and canna clusters, moreover, show the black cross between crossed nicols about as plainly as the natural granules. In the canna clusters, the contrast between the black cross and the white of the cluster is not so sharp as in the potato clusters. The other starches are merely birefringent. It is believed that the absence of the black cross is due to the small size of the clusters, for in an occasional unusually large cluster (maize) indications of a cross could be seen though not plainly enough to be certain. Moreover, in occasional very small clusters in the potato and canna preparations the black cross was impossible to see. In the potato preparations the black cross consists of two perfectly straight crossing black bands, whereas in the natural starch grains the arms of the cross are more or less wedge-shaped, with the apex of the wedge at the center of the cross—the wedges, themselves, being more or less distorted in granules that are not spherical. The arms of the cross in the canna preparations seemed to be somewhat more wedge-shaped than in the potato preparation. When a crystal cluster which is oval in outline is observed while one of the nicols is being rotated slowly, no distortion or shifting of the arms of the cross is seen as the two nicols become parallel and the black cross disappears. With a selenite plate interposed, the addition and subtraction colors in the case of the large potato crystal clusters are of the same color, and apparently of the same intensity as in natural potato

starch grains. In the case of the canna clusters, which are composed of finer needles than the potato clusters, the colors were rather purplish and orange instead of blue and gold as in the natural granules.

The observations of the writers are apparently the first on record in which from clear solutions a crystalline precipitate has been obtained, which shows optical properties very similar to those shown by natural starch. While further investigation is required to determine whether or not this crystalline substance is identical with any substance in the natural starch grain, the fact that it behaves as does natural starch when viewed between crossed nicols renders it extremely probable that the "black cross" shown by the natural starch grain in polarized light is due to crystalline structure rather than to strain or lamination.

3141

Local immunization of guinea pigs to cutaneous infection with a pasteurella isolated from wild rats.

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[*From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco, Calif.*]

In the course of the routine plague control work carried on by the U. S. Public Health Service under the direction of Dr. N. E. Wayson[1] a pasteurella infection of wild rats was encountered which seriously interfered with the rapid and accurate diagnosis of chronic rodent plague. As a rule guinea pigs cutaneously infected with the suspected tissues succumbed in 1 to 2 days to the pasteurella disease. In order to rule out mixed infections with *P. pestis* a number of special methods which will be published elsewhere were tried. It was apparent that an immunological procedure capable of protecting guinea pigs rapidly and completely against the pasteurella would probably allow the separation and

[1] Wayson, N. E., *Pub. Health Rep.*, 1925, lx, 1975.

detection of *P. pestis* in a cadaver carrying the hemorrhagic septicemia organism in the spleen, lung, etc. Passive and active immunization of guinea pigs with living avirulent or killed organisms introduced by the subcutaneous and the intraperitoneal route were tried with varying success. A detailed account of these experiments is not in the scope of this preliminary communication but attention should be called to some observations on local immunizations. Since it was found that the pasteurella passes just as readily as *P. pestis* through the carefully shaven skin it was suspected that the integumentum was the receptive organ in the sense of Besredka.[2] A series of experiments on cuti-immunization were carried out. The data are herewith briefly detailed:

Experiment No. 1: Seven strains of rat pasteurella highly pathogenic for guinea pigs were grown in shallow layers (depth 1 cc.) of beefheart-hormone broth (pH 7.0) for 18 days at 28° C. The pooled cultures were filtered through a Berkefeld V Candle under a vacuum of 15 cm. Hg. pressure. The filtrate when tested on solid and liquid mediums was found to be sterile and when seeded with young cultures of the pasteurella failed to encourage further growth. It apparently contained the "anti-virus" of Besredka. However, it was noted that, although no visible growth took place in the tests made for the demonstration of the inhibitive properties of the filtrate, the inoculated bacteria remained viable and vigorously multiplied when the seeded filtrate was diluted with hormone serum broth (20 times its volume). Six guinea pigs received intracutaneously on the abdomen 0.5 cc. of the filtrate. On the following day two animals were dead. Two of the remaining four guinea pigs, which were all sick, died on the second and the last two on the third day after the injection. Postmortem examinations only revealed a marked subcutaneous injection and gelatinous edema immediately adjacent to the site of the inoculation. Smears and cultures proved the absence of bacteria.

Experiment No. 2: A filtrate similar to that used in Experiment No. 1 was prepared. However, the cultures were grown for seven days in 200 cc. of hormone broth held in 250 cc. Erlenmeyer flasks (deep layers). The filtrate was sterile, but when seeded with the pasteurella strains gave a good culture in 24 hours. It contained no "antivirus".

[2] Immunization Locale, Paris, Masson Cie, 1925.

Eight guinea pigs were intracutaneously inoculated with 0.3 cc. of the filtrate. No reddening or swelling was evident on the following day. Twenty-one hours after the injection of the filtrate, the guinea pigs were tested with virulent splenic material containing the pasteurella organism. The tissues were rubbed into the slightly scarified skin or introduced by the pocket method into the area previously injected with the filtrate. The treated animals died as rapidly as the controls infected with the same material and in the same manner. The autopsy findings were typical for hemorrhagic septicemia and were confirmed by smears and cultures.

Experiment No. 3: The filtrate used in this experiment was prepared in the same manner as stated for Experiment No. 1, with the exception that the cultures were incubated for seven days at 28° C. It was sterile, had a pH of 6.4 and contained the "antivirus". Thirty-six guinea pigs were treated by intracutaneous injection of 0.3 cc. of filtrate into the freshly shaven abdominal skin. Twenty-four guinea pigs were injected in the same manner with broth of the identical lot as used in the production of the filtrate. At intervals of 18, 24, 48, 72 hours, 5 and 8 days, four animals treated with filtrate, four injected with broth and four healthy guinea pigs were subjected to immunity tests. The crushed spleen fragments of a guinea pig which had succumbed in 18 hours to the pasteurella infection showing innumerable typical organisms, were applied either on the mucous membrane of the nose or were smeared on the slightly scarified skin or subcutaneously pocketed in the area injected with the filtrate or rubbed on a freshly shaven zone of the back.

The most significant results, which clearly indicate that the guinea pigs intracutaneously injected with broth culture filtrates and subjected to an infection by cutaneous routes are fully protected, are shown in Table I. Since the immunity tests conducted on the 72nd hour, the 5th and 8th days furnished identical results to those presented in the table a detailed presentation is deemed unnecessary. The protection against a fatal cutaneous infection is fully developed on the 18th hour after the treatment and persists for at least three weeks (longest period thus far tested). Not only the vaccinated area but the entire skin is resistant. Furthermore, the immunity is apparently specific since the intracutaneous injection of broth conferred little or no protection. The animals treated with broth succumbed to the test

TABLE I.

Cuti-immunization—Experiment III—Immunization tested 18, 24 and 48 hours after intracutaneous injection of filtrate.

Male guinea pigs weight	Treatment injection on abdomen	Immunity tested with virulent spleen tissue		Results.
200	0.3 cc. filtrate	18 hours after test	Vaccination same area	No symptoms. Normal
195	" " "		Vaccination on back	No symptoms. Normal
210	" " "		Sub. cut. same area	Dead 45 hrs. after test
200	" " "		Smear on nose	No symptoms. Normal
180	" " "	24 hours after test	Vaccination same area	No symptoms. Normal
220	" " "		Vaccination on back	No symptoms. Normal
200	" " "		Sub. cut. same area	Dead 43 hrs. after test
210	" " "		Smear on nose	No symptoms. Normal
240	" " "	48 hours after test	Vaccination same area	No symptoms. Normal
250	" " "		Vaccination on back	No symptoms. Normal
180	" " "		Sub. cut. same area	Dead 44 hrs. after test
185	" " "		Smear on nose	No symptoms. Normal
200	0.3 cc. broth	18 hours after test	Vaccination same area	Dead 69 hrs. after test
250	" " "		Vaccination on back	Dead 72 hrs. after test
200	" " "		Sub. cut. same area	Dead 48 hrs. after test
230	" " "		Smear on nose	No symptoms. Normal
250	" " "	24 hours after test	Vaccination same area	Dead 70 hrs. after test
200	" " "		Vaccination on back	Dead 75 hrs. after test
200	" " "		Sub. cut. same area	Dead 40 hrs. after test
250	" " "		Smear on nose	No symptoms. Normal
200	" " "	48 hours after test	Vaccination same area	Dead 62 hrs. after test
220	" " "		Vaccination on back	Dead 65 hrs. after test
200	" " "		Sub. cut. same area	Dead 42 hrs. after test
250	" " "		Smear on nose	No symptoms. Normal
250	Shave abdomen only	18 hours after test	Vaccination same area	Dead 71 hrs. after test
240	" " "		Vaccination on back	Dead 75 hrs. after test
250	" " "		Sub. cut. same area	Dead 41 hrs. after test
200	" " "		Smear on nose	No symptoms. Normal
210	" " "	24 hours after test	Vaccination same area	Dead 65 hrs. after test
190	" " "		Vaccination on back	Dead 70 hrs. after test
200	" " "		Sub. cut. same area	Dead 39 hrs. after test
230	" " "		Smear on nose	No symptoms. Normal
250	" " "	48 hours after test	Vaccination same area	Dead 69 hrs. after test
210	" " "		Vaccination on back	Dead 71 hrs. after test
250	" " "		Sub. cut. same area	Dead 39 hrs. after test
200	" " "		Smear on nose	No symptoms. Normal

infection after approximately the same lapse of time (69 to 75 hours). The integumentum, even when heavily scarified and virulent material is directly rubbed into the skin capillaries, can fully ward off an infection, while the subcutaneous application of the hemorrhagic septicemia organism produces a fatal disease in the same time as in the non-vaccinated control animals (39 to 48 hours). Twelve guinea pigs intracutaneously vaccinated were bled on the 15th day and the serum tested for agglutinins

and protective substances. No serum antibodies were demonstrated. In view of the publication of Kitt[3] and others, these results were anticipated and other methods must be developed to prove more conclusively the local character of the cuti-immunity. It is reasonable to assume that the subcutaneous test inoculation (pocket method) with a pasteurella organism is too massive to demonstrate the delicate general immunity which might have developed on the 5th and 8th day. Even if such a possibility is admitted one would encounter difficulties in explaining the striking protection of the skin 18 hours after the injection of the filtrate containing a growth inhibiting substance. Experiments are in progress to shed light on the nature of the "antivirus", the duration and mechanism of the cuti-immunity.

Conclusion: Although the skin is not the main receptive organ for the bacteria of the pasteurella group, experiments conducted on guinea pigs have established the possibility of protecting the entire integumentum of the animals inside of 18 hours, but not the subcutaneous tissues, when non-toxic filtrates prepared from seven days old cultures grown in shallow layers, containing a growth inhibiting substance or "antivirus", are inoculated intracutaneously.

3142

Changes in the alveolar process about the teeth in dogs on experimental diets.

MARTHA R. JONES and F. V. SIMONTON. (Introduced by T. D. Beckwith).

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Studies on inorganic salt metabolism in dogs conducted by one of us (M. R. J.)[1] have shown that skeletal changes and dental defects may be induced in normal puppies on diets which appear

[3] Handbuch d. pathogen. Mikroorgan., 2nd Ed., 1913, vi, 56.

* Aided by a grant from the W. H. Crocker Fund for research in pediatrics.

[1] Jones, Martha R., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 199.

to be adequate in respect to protein, fat, carbohydrate, inorganic salts and vitamins (bread, meat, milk, butter fat, orange juice and salt mixture based on ash analysis of milk), but which contain an excess of basic ions through additions of sodium carbonate. To date, microscopic sections have been prepared from

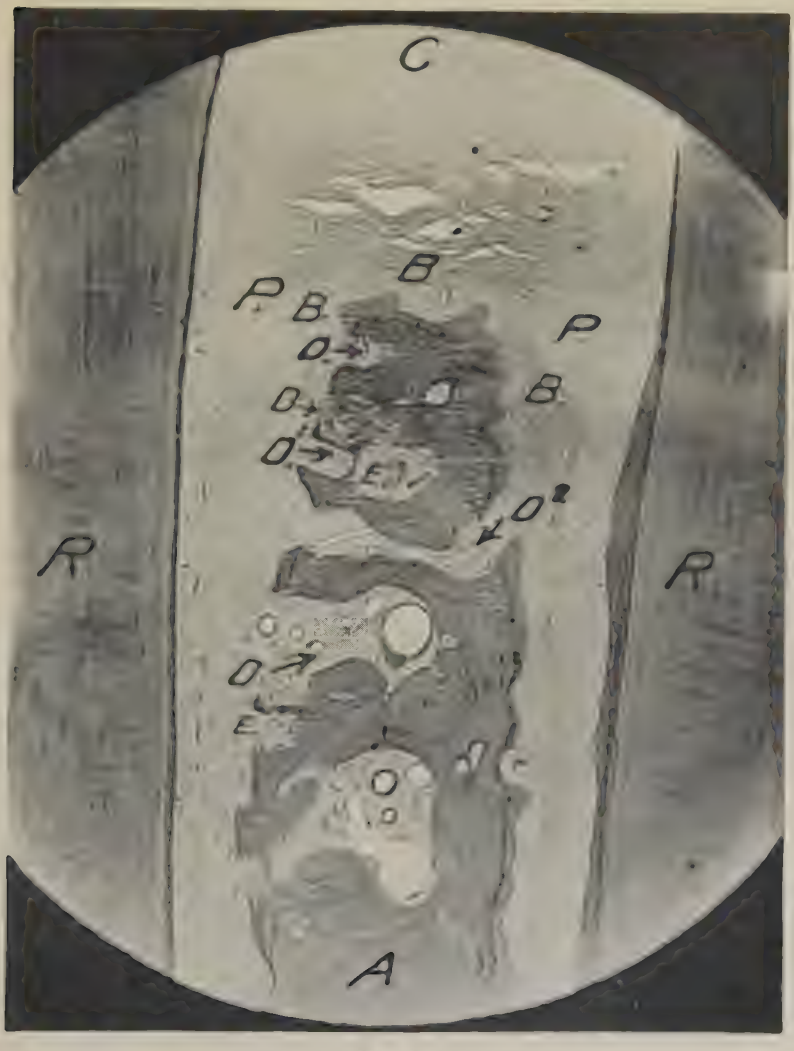

FIG. 1.

Retrograde changes in the alveolar process in an adult dog on diets which appear to be adequate in respect to protein, fat, carbohydrate, inorganic salts and vitamins, but containing an excess of basic ions.

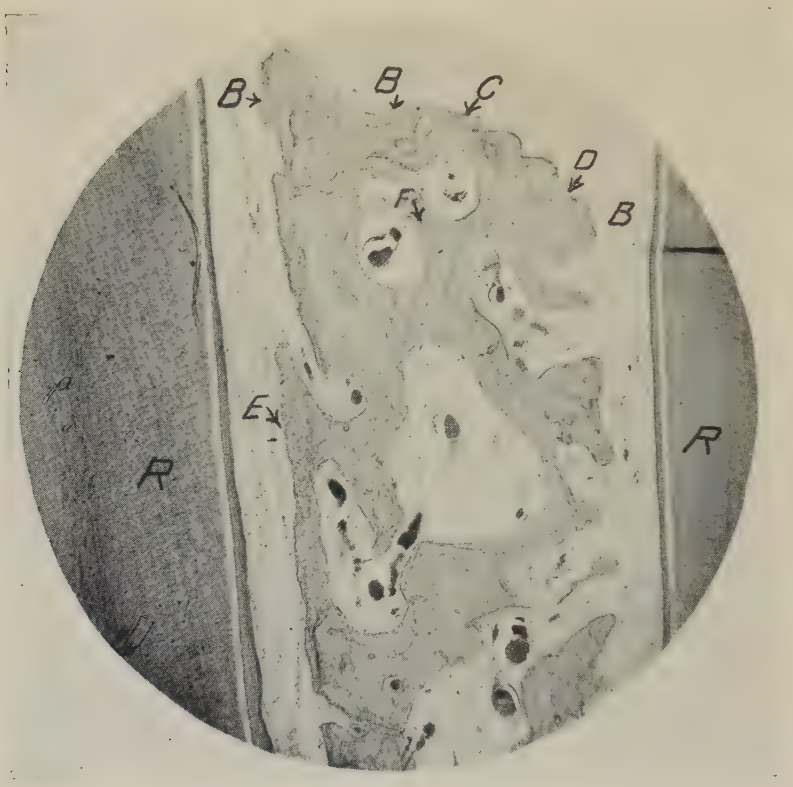

FIG. 2.
Changes in the alveolar process, corresponding section, from the same animal, subsequent to a change in diet in which the soda was omitted and rice substituted for potato, resulting in an excess of acid ions.

six of these puppies and in all cases show retrograde changes in the alveolar process. Marked clinical evidence, such as loosening and migration of the teeth and softening of the bone of the process to the extent that the tissues can be excised with a knife, exists in other cases from which microscopic sections are now being prepared. Inquiries into the dietary preferences of a number of persons suffering from parodontoclasia[2] have shown that alveolar atrophy occurs, in the majority of cases, on diets which are relatively low in protein and minerals and contain a preponderance of base. In the light of these observations, a group of normal adult dogs, ranging in age from 3 to 7 years,

[2] Simonton, F. V., *Dental Cosmos*, February, 1926.

were placed on diets consisting essentially of bread, meat and potato—the latter predominating. In certain cases beef suet was added to the food mixture, in others, sodium carbonate to further increase the alkalinity. The case reported below was one of two animals placed on the diet as described with the addition of sodium carbonate. In spite of the high potential alkalinity of the food mixture, both animals excreted urines which varied around the neutral point (pH 7.0)—the amount of soda having to be continually increased in order to maintain the desired urinary reaction. After 130 days on the alkaline diet the upper second and third incisors were removed at biopsy from both animals. Histologic sections, one of which is shown in Fig. 1, indicated retrograde changes in the alveolar process. These were quite comparable in the two dogs. At the time of biopsy, the animals were robust, well nourished, and appeared to be in good health. Referring to Fig. 1, the roots of the upper second right and third incisors are shown at *R* and *R*. *C* lies in the direction of the crowns, *A* toward the apices of the roots. The septum of the alveolar process shows marked retrogressive changes. Halisteresis has occurred at *B-B-B*. Lacunar resorptions are evident at *D-D*. At D^1 some resolution with fibrosis has occurred. At D^2 a complete division of the septum (in the plane of this particular section) has occurred. Osteoporotic changes are evident in the marrow spaces. Multinuclear osteoclasts may be seen at *E-E*. Widening of the peridental membrane is apparent at *P-P*. This results from the narrowing of the septum through resorptive changes. The freedom of the soft tissues from any evidence of inflammatory processes is notable.

Subsequent to the biopsy, omission was made of the soda from the food mixture during the following 48 days, resulting in a prompt increase in the acidity of the urine, the pH dropping as low as 6.6, in spite of the potential alkalinity of the diet. Reversal of the dietary reaction by the substitution of an equivalent amount of rice for the potato resulted in a further increase in urinary acidity during the first few days. Subsequently, both animals went into convulsions and died.[3] The one reported, died on the 28th day after the change in diet. Corresponding tissue (the upper left second and third incisors, Fig. 2) was removed post mortem.

[3] Jones, Martha R. (In press.)

Referring to Fig. 2, marked reconstructive changes are evident. The roots of the upper left second and third incisors are shown at *R* and *R*. The subperiosteal bone *(B-B)* is fairly abundant. Along the crest *(C)* new bone formation is taking place. Under higher magnification, osteoblasts may be seen being embedded along the margin as bone corpuscles, many appearing between the margin of the bone and the outlying blood vessels. The superficial bone corpuscles are large and their nuclei regular in outline and clearly defined. At *E* may be seen old dead bone overlaid by new. At *F* two marrow spaces which had previously coalesced have been separated by bone growth between them.

Sections from the long bones showed no apparent recent changes of the osseous tissue. Complete histologic studies of the entire group of animals are in course of preparation and will be given in detail later.

SUMMARY.

1. The condition shown in Fig. 1 represents a non-inflammatory type of parodontoclasia in a dog.

2. The occurrence of the retrograde changes in Fig. 1 in relation to a diet known to produce osseous degeneration, together with a reversal of the type of bone change with a reversal of the dietary fault, postulates that nutrition is an important etiologic factor.

3. The microscopic appearance of the tissues shown in Fig. 1 resembles diffuse atrophy in human tissues as described by Gottlieb.[4]

4. The condition described as resulting from the basic diet occurred without any obvious degenerative changes in the long bones and in an animal apparently in good health, robust and well-nourished. This is in conformity with the occurrence of parodontoclasia in humans who seem to be in perfect health.

5. The freedom from inflammatory reaction of the soft tissues may be due in part to the high resistance to infection characteristic of the tissues of the dog, and in part to lack of function. The dogs were fed on pap, requiring practically no mastication. It is possible that vigorous use of the tissues in which the skeletal support was so defective would lead to trauma, hemorrhage, and invasion of micro-organisms.

[4] Gottlieb, B., *Zeitsch. fur Stomat.*, 1923, iv, 195.

6. The bone changes represented in Fig. 1, namely, resorption of the crest of the alveolar septum, lateral excavations, opening of the bone marrow spaces to the side, osteoporosis, coalescing of marrow spaces and widening of the sockets of the teeth crownwise, all compare with the appearances familiar to parodontists in roentgenographs of human parodontoclasia.

Missouri Branch

Washington University Medical School, April 21, 1926.

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The blood volume in cases of nephritis with edema and low serum protein concentration.

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Although a fairly large number of determinations of the blood volume have been made in cases of chronic nephritis with hypertension, very few are reported in cases of parenchymatous nephritis (nephrosis). Plesch,[1] using his infusion method, reported one case in 1909 in which the blood volume was about half the normal amount. Using the carbon monoxide method, Plesch[2] reported in 1922 a less marked decrease in the blood volume in one patient. In this paper, he also reported two cases of chronic nephritis with edema, which showed increasing blood volumes with decreasing edema. Linder, *et al.*,[3] used the dye method on a number of cases of nephritis. They found slightly low blood volumes in two cases of parenchymatous nephritis.

The following cases are reported because of the strikingly low figures found. In all but one case, more than one determination was made.

Blood volumes were determined by the method of Keith, Roun-

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[1] Plesch, J., *Z. fur Klin. Med.*, 1922, xciii, 241.

[2] Plesch, J., *Z. exp. Path. u. Therap.*, 1909, vi, 380.

[3] Linder, G. C., Lundsgaard, C., Van Slyke, D. D., and Stillman, E., *J. Exp. Med.*, 1924, xxxviii, 921.

tree and Geraghty.[4] Three of the cases were typical instances of parenchymatous nephritis in every respect. One case (No. 2) was not entirely typical as the N. P. N. was elevated and blood cholesterol was normal. Otherwise the blood of all the cases showed high cholesterol concentration, and only slight or no elevation of the non-protein-nitrogen. The urine of these patients was concentrated, showed large amounts of albumin, casts and only occasional red blood corpuscles. There was no elevation of blood pressure. Patient No. 2, on admission, was suffering from pneumonia, as well as chronic nephritis. This patient's urine was of high specific gravity, showed large amounts of albumin, but no red blood corpuscles. His whole blood non-protein nitrogen concentration was 115 mg. per 100 mm. and the whole blood cholesterol was 155 mg. per 100 mm. He died during a later attack of pneumonia and no permit for necropsy could be obtained.

The recent studies of Smith[5] have shown that the vital red method of determining blood volumes is reliable for comparative studies. However, the results are not strictly comparable to those obtained by the carbon monoxide method. The latter method apparently gives figures a little too low because of the uneven saturation of the red corpuscles with carbon monoxide, while the former method measures an uncertain amount of lymph. Therefore, the method of Keith, Rountree and Geraghty tends to give slightly high results.

The findings on our cases are given in the accompanying table. From other studies of the blood volume, which are to be reported later, it was found, as would be expected, that the plasma volume is much more constant than the whole blood volume. By the method used, children over 18 months old who have a normal water metabolism, show plasma volumes of approximately 50 mg. per kilogram of body weight. Only in severe anemia does there seem to be any great variation from this figure, unless either dehydration or edema be also present. Adults tend to show the slightly lower figure of 45 mg. per kilogram. However, the results are more convincing when one compares the different figures on the same patient during different states of his water metabolism, as manifested by varying amounts of edema and absence of edema.

[4] Keith, N. M., Rountree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547.

[5] Smith, H. P., *Bull. J. H. H.*, 1925, xxxvi, 325.

	Wt. kg.	Age Yrs.	N. P. N. mg. per 100 mg.	Tot.W.B. Cholest'l mg. per 100 mg.	Cell Vol. %	Serum Protein % | gm./kg.	Gm. K.	Blood Volume						Edema
								W. B. mg.	Pl. mg.	Cells. mg.	*WB* kg.	*PL* kg.	*Cells* kg.	
IX-25-24 Case I.	15.1	2¾		350	28.3	7.09	2.18	649	465	184	43.	31	13	+++
Parenchymatous nephritis														
I-9-25 Case I.	15.02	3	40	247	32.7	7.37	2.01	609	410	199	40.5	27	13	+++
I-13-25 Case I.	15.00	3			30.0	7.09	2.2	614	430	184	41.	28	15	+++
V-13-25 Case I.	14.5	3¼	47	250	31.3	8.55	4.26	1054	724	330	72.5	50	22.5	0
XII-2-24 Case II.														
Subacute nephritis with edema and Ascites	40.45	11	84.5	155	34.4	6.62	2.46	2252	1500	752	55.7	37.2	18.6	++++
XII-18-24 Case II.	39.77	11	115		25.5	6.22	1.85	1586	1182	404	40.0	30	10	++++
IV-4-25 Case III.														
Parenchymatous nephritis	60.95	39			38.	6.34	2.7	4200	2605	1595	69.	41	26	++
III-9-25 Case IV.														
Parenchymatous nephritis	33	9			57.7	6.34	1.99	2080	880	1200	63.1	26.6	36.4	+++
IV-22-25 Case IV.	31	9			49.5	5.99	2.38	2437	1230	1207	75.	38.6	39	++
V-5-25 Case IV.	27.7	9			49.3	6.39	3.04	2600	1320	1280	93.8	47.6	46.2	0

Serum protein per cent determined with Abbe refractometer.
Edema ++++ generalized edema with ascites.
Edema +++ generalized edema.
Edema ++ pitting edema of dependent parts and puffiness of face.
Edema 0 no perceptible edema.

All the patients while edematous showed marked decreases in their plasma volumes, and all except patient no. 4 had decreases in the cell volume as well. The low serum protein concentration in this type of case is well recognized, but the low plasma volume, together with the low serum protein concentration, indicates a greater total loss of plasma protein than has hitherto been suspected. The average amount of serum protein per kilogram of body weight, during the stage of edema, was 2.2 gm. This is about half the amount present in normal children, and agrees with the figure of Linder and others.[3] It is to be noted that, with recovery, the plasma volume returns to normal and that there is an absolute decrease in the plasma volume from that found when no edema is manifest. The amount of plasma per kilo is reduced, if one uses the edema-free weight as well as the weight, during the determination. The figures indicate that the patients of this type usually are suffering from an erythropenia far greater than any red cell count or hemoglobin determination could detect.

The methods used in this study do not give any indication as to whether part of the plasma proteins are removed to other tissues, or whether all the loss occurs by way of the urine. These data would suggest a more frequent use of transfusions than is now practiced, to replace both the plasma proteins and the red cells.

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The influence of posture on renal activity.

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The influence of posture on the renal output of water, bicarbonate, chloride, inorganic phosphate, inorganic sulphate, urea, ammonia, creatinine and titratable acid, on urine pH, on blood pressure, pulse rate, circulation rate and rate of metabolism has been studied. The procedure was as follows: The subject took no

TABLE I.
Averages of all experiments on each subject.

Subject	Posture	Urine cc. per hr.	CO_2 mg. per hr.	NaCl mg. per hr.	P mg. per hr.	S mg. per hr.	Urea N mg. per hr.	Ammonia N mg. per hr.	Creatinine mg. per hr.	Titratable acidity cc. N/10 per hr.	pH	Spec. Grav.
H 8	S	51	5.5	362	14.9	19.6	262	27.4	56.5	6.1	5.4	1.016
	R	185	37.6	862	22.1	26.3	457	28.3	60.5	7.5	6.0	1.009
	per cent increase in recumbent	263	584	138	48	34	75	3	7	23		
D 6	S	50	2.2	296	14.4	18.3	386	32.8	47.7	9.3	5.0	1.015*
	R	103	5.8	468	21.8	22.6	488	25.1	52.8	12.6	5.1	1.012
	per cent increase in recumbent	106	164	58	51	23	26	—23	11	36		
S 4	S	35	3.8	220	18.4	10.2	166	11.7	41.7	6.2	5.6	1.016
	R	155	38.0	603	27.8	16.8	319	11.2	46.8	6.7	6.0	1.006
	per cent increase in recumbent	343	900	174	51	65	92	—4	12	8		
Average increase for all subjects in recumbent		237	549	123	50	41	64	—8	10	22		

*Specific gravity not determined in one standing sample of 11.7 cc.

Each standing figure and each recumbent represents for subject H the average of eight periods, for D of six, and for S of four.

food or water after ten p. m. of the evening preceding an experiment. At eight a. m. he emptied the bladder, discarding the urine, drank 200 cc. of tap water and stood or lay for two hours, at the end of which he voided, drank another 200 cc. of water and lay or stood for another two hour period. At the end of each two hour period he emptied the bladder and drank another 200 cc. of water. The order of lying and standing periods was varied in the various experiments. Each experiment was continued for four consecutive two hour periods. Nine experiments were performed on three subjects, four on subject H, aged 18 years, three on subject D, aged 30, and two on subject S, aged 50. The circulation rate for a ten minute period was determined by Henderson's and Haggard's method[1] in at least one lying and one standing period. During the period of circulation rate determination, ten arterial blood pressure readings were made, using the auscultatory and graphic (Erlanger instrument) methods simultaneously. Each blood pressure and pulse rate figure given in the table is thus the average of ten readings. No food was taken during the experiment and no water except the 200 cc. at the beginning of each period.

The circulation rate, blood pressure and metabolism figures are not presented because of lack of space. In every case the general circulation rate was greater in the recumbent posture and in all the experiments except those on subject S the mean arterial blood pressure was lower in the recumbent. The table gives the averages of the urine data of all the experiments on each subject. The complete data and a discussion of their bearing on the question of the mechanism of renal activity will appear in a later communication. We believe that the variations in the output of water and of the urine solids are in part explained by the view that the changes of posture vary the number of glomerular capillaries exhibiting an active circulation, the number being increased in the recumbent posture.

[1] Henderson, Y., and Haggard, H. W., *Am. J. Physiol.*, 1925, lxxiii, 193.

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The relation of pulse pressure to stroke volume.

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In 1904 Erlanger and Hooker[1] suggested that the product of the pulse pressure (P. P.) and the pulse rate (P. R.) might be used as an index to the circulation rate (C. R.), or, expressed differently, the pulse pressure as an index to the stroke volume (S. V.), provided changes in systolic time and elasticity of the arteries do not disturb too much the direct relation of the P. P. to S. V.

In order to ascertain what the relation is, four normal individuals were studied under four relatively normal conditions. The conditions studied were the recumbent and standing postures and light and post-heavy exercise. Arterial pressure determinations could not be made during heavy exercise; therefore, they were made immediately afterwards.

The arterial pressure readings were made simultaneously by the oscillatory (Erlanger) and the auscultatory methods. The circulation rate in liters per minute was determined by the Henderson and Haggard[2] method, involving the inhalation of ethyl iodide. Ten arterial pressure determinations were made to each observation of the circulation rate and the average of these are used as a basis of comparison.

When the S. V. is plotted against the P. P. it is found that a straight line passing through the points indicating the mean values for standing and light exercise almost, if not quite, passes through zero and also the mean of heavy exercise, indicating that the relationship between these is one of direct proportionality. On the other hand the mean of the recumbent observation always falls to the left of this line. The recumbent pulse rate and diastolic pressure are in each case decidedly lower than in the other states. The longer systolic time of the slower P. R. and the re-

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[1] Erlanger, J., and Hooker, D. R., *The Johns Hopkins Hospital Reports*, 1904, xii, 147.

[2] Henderson, Y., and Haggard, H. W., *Am. J. Physiol.*, 1925, lxxiii, 193.

Subject		Averages						
		P. R.	D. P.	P. P.	S. V.	*S. V.* / P. P.	P. P. × P. R.	C. R.
T. H.	Recumbent	72.1	72.5	45.3	82	1.79	3279	5.85
	Standing	95.5	86.9	25.3	39.5	1.56	2471	3.72
	Lt. Exercise	108	79.3	46.6	78	1.66	5063	8.4
	Post-Hvy Ex.	131	86	73	131	1.83	9882	16.7
W. D.	Recumbent	64.6	62.2	45.6	108	2.42	2889	7.06
	Standing	82.8	71.8	33	58	1.77	2716	4.78
	Lt. Exercise	84.5	63	53	96	1.805	4313	8.1
	Post-Hvy Ex.	128	64	85	146	1.72	10090	18.7
E. E.	Recumbent	64.5	86	43.5	86	1.97	2785	5.5
	Standing	93.5	88.5	33.2	46	1.38	3108	4.32
	Lt. Exercise	88	92.5	48	76	1.58	4235	6.7
	Post-Hvy Ex.	93	87	64	103	1.58	5820	9.65
D. W.	Recumbent	69	72	40.5	105	2.59	2795	7.25
	Standing	100	73.5	35	48	1.37	3523	4.8
	Lt. Exercise	103.5	72	57.5	93	1.62	5960	9.7
	Post-Hvy Ex.	117	71	85	129	1.51	10025	15.1

duced coefficient of elasticity associated with the lower diastolic pressure both would have the effect of reducing the P. P. produced by a given stroke volume, and both would, therefore, tend to put the mean of the recumbent reading to the left of the line passing through the mean standing and exercise readings.

The scattering of the points and the fact that the lines drawn through the mean of the standing and exercise determinations do not pass through zero must be attributed to the influence of systolic time and arterial elasticity upon the relation to P. P. to S. V. It so happens that these modifying factors do not, in our observations, materially obscure the linear relationship of P. P. to S. V. The scattering of the points around their respective means is in part explained by experimental error, and in part again by the influence of systolic time and arterial elasticity. Thus the determinations in which the pulse rate is relatively rapid all tend to fall to right of the line.

Obviously when the product P. P. $\times$ P. R. is used as an index to the C. R. the modifying influence of the systolic time on the relation of P. P. to S. V. will have to be taken into consideration. In every case save two, the means of the P. P. $\times$ P. R. and of the C. R. change in the same direction. The two exceptions occur in the change from the recumbent to the standing posture and in these two cases the pulse has the largest rate.

3146

The action of adrenalin on the pyloric sphincter.

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Because of the lack of consistent data bearing on the action of adrenalin on the pyloric sphincter, a further investigation of the problem has been undertaken. The results so far obtained have been sufficiently constant that a preliminary report, indicating their general character, seems justified. The investigation has been confined to a study of the immediate effects of the drug administered intravenously to anesthetized dogs. Several methods

have been employed to observe the reactions of the sphincter muscle without significant differences in the results.

In dogs that have been anesthetized with morphin and ether, and allowed an hour or more for recovery from operative procedures, adrenalin produces regularly a prompt relaxation of the sphincter muscle. After a variable interval, the duration of which depends on the dosage, the tonus recovers and may temporarily reach a level slightly higher than before the drug was given. On the other hand, if observations are made immediately after operating on the animal or if ether alone is used for anesthesia, the administration of adrenalin usually causes, immediately, a slight increase in the tonus of the sphincter.

No very pronounced changes in the tonus of the sphincter have been observed as a result of administering adrenalin. The greatest increase in tonus obtained with adrenalin was much less than that which results from morphin, or from vagus stimulation. When a decrease in tonus is obtained it is generally somewhat more pronounced, but is decidedly less than is seen in the small intestine when adrenalin is given.

Although Gruber[1] and others have found that adrenalin may contract or relax gastrointestinal muscle, depending on the dosage, no such influence of dosage is apparent in the results of these experiments so far, but further study of this point seems desirable. Under the experimental conditions that have obtained in this work up to the present, identical doses produce opposite effects in the same animal at different times, and under certain conditions, qualitatively similar results are obtained with a wide range of dosage. Larger doses (*e. g.*, 0.05 mg. per kilo) produce a somewhat more prolonged effect than smaller ones, (*e. g.*, 0.005 mg. per kilo) but an inhibitory response was not observed to change to motor, or vice versa, as a result of change of dosage.

On the other hand, a sphincter muscle which is responding constantly to adrenalin with an increase in tonus can be made to change its response to relaxation by procedures which increase the tonus of the muscle, *e. g.*, the administration of morphin or stimulation of the vagus, the adrenalin being given during the period of stimulation. This fact suggests an explanation for the different responses obtained under different conditions. In animals under ether anesthesia without morphin, and in animals

[1] Gruber, Charles M., *J. Pharm. and Exp. Therap.*, 1922, xx, 321.

recently subjected to operative procedures the tonus of the sphincter is found to be comparatively low. It is under such conditions that motor effects are obtained. On the other hand the records indicate a gradual recovery of tonus in the sphincter muscle after operation if the animal is not disturbed, and a sudden and permanent increase in tonus if morphin is administered. When these conditions are established the response to adrenalin is characteristically inhibitory.

It appears, therefore, that adrenalin increases the tonus of the pyloric sphincter when the muscle is relaxed and decreases it when the muscle is contracted. It will be recalled that these results correspond to those described by Carlson and Litt[2] as following stimulation of the sympathetic nerve supply.

3147

The conjugation of benzoic acid in rabbits.

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A study has been made of the excretion of free benzoic acid, hippuric acid, and total combined benzoic acid in the urine of normal adult rabbits following the administration of sodium benzoate. The maximum rate of synthesis of hippuric acid occurred after the ingestion of 0.5 gm. of benzoic acid per kilo. Larger doses of benzoic acid increased the rate of excretion of combined benzoic acid but not that of hippuric acid. In the 24 hour period following the administration of 1.0 gm. of benzoic acid per kilo, the average excretion of hippuric acid in six rabbits was 82 per cent of the combined benzoic acid, the individual variations ranging from 65 to 90 per cent. These urines contained an ether-soluble, non-fermentable reducing substance which gave a positive naphtho-resorcin test. Therefore, it was concluded that 10 to 35 per cent of the combined benzoic acid excreted by the six rabbits was benzoyl glycuronic acid. It has

[2] Carlson, A. J., and Litt, S., *Arch. Int. Med.*, 1924, xxxiii, 281.

long been known that the rabbit excreted benzoyl glycuronic acid following benzoate administration, but it has generally been assumed that this substance was present only in traces. These experiments indicated that the extent to which the rabbit detoxicates ingested benzoate by conjugation with glycuronic acid has been underestimated.

Illinois Branch

University of Chicago, April 27, 1926.

3148

The experimental production of achylia gastrica in the dog.

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Bylina,[1] studying pancreatic secretion, applied hot water (70° R.) to the gastric mucosa in order to produce a temporary achylia. An achylia, which lasted from six to seven days, resulted from one application.

It occurred to us that permanent achylia might be produced by application of hot water at intervals. Our method has consisted in injecting into the stomach via a gastrostomy from 250 to 300 cc. of water at 70° to 75° C., allowing it to remain in the stomach for one minute. This has been repeated at six to ten day intervals. The gastric secretory response to a meal, 1 mg. of histamine and 2 mg. of histamine, has been followed.

Our experiments have been under way for two months. We have found that the gastric glands neither respond to a meal, nor to 1 mg. of histamine. In some tests, in which we used 2 mg. of histamine to excite the gastric glands, one week after the last application of water, we obtained from 5 to 10 cc. of mucous which contained free acid. We find that it is difficult to maintain the nutrition of these animals, which difficulty we hope to overcome.

Our observations show that hot water, lower in temperature than that used by Bylina, can be used to cause a temporary achylia, and that the achylia can be maintained by intermittent applications of the hot water at from six to ten day intervals.

[1] Bylina, A. S., *Babkin, Aüssere Sekretion der Verdanungsdrusen*, page 274, Berlin, 1914.

3149

A demonstration that a hormone is concerned in external pancreatic secretion.

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A dog with the tail of the pancreas transplanted under the skin, and a Thiry fistula of the jejunum was demonstrated. The continuous secretion of the pancreatic transplant was collected for two hours. It amounted to 0.4 cc. Normal twentieth hydrochloric acid was applied by perfusion to the mucosa of the Thiry fistula after washing it with warm 0.9 per cent salt solution. After a latent period of 5 minutes a copious flow of juice from the pancreatic transplant resulted, amounting to 0.5 cc. in twenty minutes.

This experiment clearly demonstrates that when dilute hydrochloric acid (from N/10 to N/20) is applied to the intestinal mucosa something (a hormone, we believe) is caused to enter the blood or lymph stream which excites the cells of the pancreas to form pancreatic juice.

3150

Blood sugar during the crisis of malarial fever.

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In previous papers[1] we have called attention to the fact that a distinct balance exists in the autonomic orientation between the peripheral region of the body and the splanchnic area. Muller[2] has studied the relation of this balance to the distribution of

[1] Petersen, W. F., Levinson, S. A., and Hughes, T. P., *J. Immunol.*, 1923, viii, 323-407; Müller, E. F., and Petersen, W. F., *Kl. Wchr.*, 1926, v, 2.

[2] Müller, E. F., *Arch. Int. Med.*, 1925, xxxv, 796.

leukocytes, while in our own work with the lymph we have studied the alternations in permeability. In the present series of observations we have studied the changes in the blood sugar during malarial fever (artificial inoculation) with particular reference to the crisis.

In the fasting patient we have determined that the period of the chill and rise in temperature is associated with a decrease in the blood sugar level. This corresponds to the time of a peripheral leukopenia and a diminished concentration of blood protein. With the crisis and fall in temperature, blood sugar increases, leukocytes increase in the peripheral blood stream and the blood proteins increase. These pictures can be regarded as a result of a reversal of the autonomic balance. In the first stage we have a peripheral sympathetic tonus with a splanchnic parasympathetic orientation; in the period of defervescence, a peripheral parasympathetic and splanchnic sympathetic tonus. We cannot, of course, exclude the effect of metabolic utilization of the sugar. We believe, however, that the magnitude of the sugar mobilization during the period of defervescence would indicate a splanchnic sympathetic effect.

3151

The culture of planarian tissues in vitro.

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This preliminary paper is devoted to a description of the technique and some suggestive results of the culture *in vitro* of the tissues of the flatworm, *Planaria dorotocephala.* Practical sterilization of the material has been accomplished by exposing the worms, in a small amount of water, to ultra-violet radiation from a quartz mercury arc lamp at a distance of 35 cm. during 4 minutes. After such an exposure the bacteria associated with the worms are practically all killed or prevented from reproducing, without injury to the worms. The hanging-drop, as well as the petri-dish technique were used for the tissue cultures.

Buffer solutions composed of the common physiological salts,

in order to be tolerated by the Planarian explants, must be between 1/8 and 1/25 the concentration of chick serum. The optimum concentration of Planaria is 1/10 to 1/12 the chicken concentration. Intact worms, however, tolerate indefinitely all concentrations between well-water and buffer solutions containing from 1/4 to 1/5 the salt-content of vertebrate-isotonic solutions. The organ of osmotic regulation seems to be the external epithelium of the worm.

In hanging-drop cultures the cells survive in fluid media for as long as 10 to 15 days; they are pseudopodially active, and show some cell division, but not active, sheet-like proliferation. When support and tension are afforded to the explant by the addition of a drop of agar, a conspicuous sheet-like outgrowth of parenchyma may be seen within 12 hours. The cells will migrate along fine silk strands, but do not spread in sheets. Under certain conditions, cells in petri dish cultures may survive as long as 64 days.

Serum or tissue extracts of the following animals have been introduced into the culture media: tapeworm, clam, snail, isopod, frog, sheep, without injurious effect upon the explant. Sheep serum is stimulating. The artificial media used are buffer solutions to which are added a dextrose, dextrose and peptone, glycogen, leucine, arginine and tyrosine, respectively. Dextrose or glycogen prolongs the life of the cells considerably beyond their time in a buffer solution alone. The mixture of dextrose and peptone perceptibly stimulates them to migration and division. The amino-acids used are unfavorable, leucine least so.

The various types of cells have been identified in culture, and their behavior studied.

The size of a typical explant is about 1/120, or less, the volume of a whole worm. When such pieces are introduced into hanging drop conditions, a variable percentage of them may fail to spread out amorphously in the form of tissue cultures, and may round up and form individuals, some of them obviously polar, and some of them such that no polarity is discernible. The polar individuals are the more viable. One apolar individual is recorded to have changed its behavior to that of the polar type; many times, the polar type has been observed to disintegrate rather suddenly, and one or more of the apolar type to form subsequently from the debris. Apolar individuals may form originally from fragments of the explant.

3152

The presence of insulin in chicken tissues.

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Koppanyi, Ivy, Tatum and Jung[1] recently found that when the pancreas of the chicken is removed, a Von Mering-Minkowski diabetes mellitus is present for seven or eight days only. After this period the blood sugar returns to normal and sugar disappears from the urine.

Since it was not known whether or not the pancreas and other tissues of the chicken contained insulin, it was decided to assay some of the tissues of the normal chicken for insulin content. Such knowledge would be necessary before one could arrive at any interpretation of the insulin content of the tissues of depancreatised chickens. Also, this question is involved in any explanation that might be offered for the above strange and interesting observation.

Through the co-operation of Dr. Jones and Mr. Templeton of the Research Division of Swift and Company, we were able to remove the pancreas, liver and kidneys from one hundred chickens within one hour after death.

Insulin was prepared from these tissues by Fisher's[2] modification of the Doisy-Shaffer[3] method.

Three hundred and thirty grams of pancreas, 240 grams of kidney, and 712 grams of liver were extracted, the liver containing a high percentage of lipins was more difficult to work with. After filtering off the toxic portion, the insulin was precipitated, dried, weighed and made up to the following volumes: 1.8 gram of precipitate from the pancreas was made up to a volume of 42 cc. 1.5 gram of precipitate from the kidney and 1.8 gram of precipitate from the liver were dissolved in 52 cc. of distilled water respectively. The solutions were preserved with cresole.

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[1] Koppanyi, T., Ivy, A. C., Tatum, A. L., and Jung, F. T., *Am. J. Physiol.*, lxxvi, 212, 26.

[2] Fisher, N. F., *Am. J. Physiol.*, 1923, lvii, 57.

[3] Doisy, E. A., and Shaffer, P. A., *J. Biol. Chem.*, 1923, lv, 31.

In order to determine the strength of the insulin preparations different amounts were injected into rabbits. The rabbits had been starved for 24 hours.

The following tables illustrate some typical results obtained:

TABLE NO. I—PANCREAS.

4-16-26.

5 cc. pancreas at 10:30.

Normal 9:15	0.101
11:10	0.041
Convulsions at 11:10	
Injected 20 grams of glucose	
Injected again at 2:00	
Injected again at 5:00	

TABLE NO. II—LIVER.

3-4-26.

5 cc. liver at 2:00.

Normal 2:00	.125
3:00	.0454
4:00	.044
5:00	.048
6:30	.068

TABLE NO. III—KIDNEY.

3-6-26.

10 cc. kidney injected at 10.00

Normal 10:00	0.13
12:00	0.108
1:15	0.10
3:15	0.050
5:00	0.068

TABLE NO. IV.

Lilly insulin injected at 10:15.

3 cc. of U 10		2 cc. of U 10	
Normal 10:00	.116	Normal 10:00	0.12
12:00	0.053	Convulsions at 11:40	
1:15	0.050	Blood at 11:40	0.041
5:00	0.048	Given sugar, recovered	

By comparing the above results it will be noticed that 5 cc. of the solution prepared from the pancreas produced convulsions and lowered the blood sugar of a rabbit to 0.041 in two hours. Five cc. of the pancreas solution was found to be equal to 30 clinical units of Lilly's insulin, under the same conditions. The yield of insulin from the chicken pancreas calculated in terms of units per kilo of pancreas would be equal to 760. Since 12.5 cc. of the kidney solution produced similar results to 5 cc. of the pancreas, the yield of insulin per kilo of kidney is 554 units. 7.5 cc. of the liver solution was found to be equal to 5 cc. of the pancreas. The yield of insulin from the liver, per kilo of liver, would be equal to 295 units. These comparisons are, of course, rough because we had only small quantities of material with which to work.

These results show that the pancreas of the chicken contains approximately as much insulin as has been reported to occur in the pancreas of calves and that the kidney and liver of the chicken contain relatively large amounts of insulin.

3153

High and low protein diets and excretion of nitrogenous compounds in normal and undernourished children.

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[*From the Elizabeth McCormick Metabolism Fund of the Nelson Morris Institute of the Michael Reese Hospital, Chicago, Ill.*]

The work presented is part of a series of investigations of the metabolism of undernourished children of school age, who showed no pathological complications on medical examination, except that they were underweight for their height. The normals were not only normal in weight, but were healthy in every respect.

In this study the children were put first on a high and then on a low protein diet of at least 6 days each, and for the last 3 days 24-hour specimens of urine were collected and analyzed for total nitrogen, ammonia nitrogen, creatinine, and creatine. The nitrogen of the foods was also determined.

There is no marked difference in nitrogen absorption in the two groups. Both of them absorbed more nitrogen on a high protein diet, although the percentage absorption was lower. Ammonia excretion was decidedly higher in the undernourished children both computed in terms of mg. per kilo of body weight and in percentage of total nitrogen. This high ammonia excretion is probably due to the greater demand for alkali for neutralization of the excessive fatty acids produced in the intestines and eliminated as soaps in undernourished children. The alkali loss in the tissues is made up by ammonia formed in the body and excreted in the urine.

Creatinine excretion is independent of protein intake and there is no difference in the amount excreted by the two groups of children. On the other hand creatine seems to vary with protein intake in both groups, although the undernourished children excrete more creatine on low protein diet than the normals. The ratio of creatine nitrogen to total nitrogen output is remarkably constant. The higher creatine excretion in the undernourished children on a low protein diet suggests that they excrete more endogenous creatine.

3154

Effect of reticulo-endothelial blockade on agglutinin formation.

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[*From the Albert Kuppenheimer Fund of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research, Chicago, Ill.*]

The reticulo-endothelial system is considered at present to be the possible place of antibody formation. Several observers[1] have offered proof that the physical blocking of this system diminished the production of hemolysin and precipitin. Reports, however, differ on the effect of blocking the system on the production of agglutinin. In our experiments, therefore, we have limited our observation to the effect of reticulo-endothelial blockade on typhoid agglutinin formation in rabbits. Colloidal iron, a saccharated iron-oxide solution, 35 grams per 100 cc. of water, was used for the indifferent non-protein material. It was injected intravenously in 2 to 6 cc. amounts, depending on the weight of the rabbit, almost daily until nine to nineteen injections were given. In most of the rabbits the iron was given preliminary to the typhoid vaccination. In several rabbits, however, it was continued throughout the period of immunization; in several rabbits the antibody titre was permitted to drop, and then the iron injection resumed.

The rabbits stood the iron injections well, and there was no loss in weight. The blood picture was little changed; the hemoglobin and red blood cells were practically unchanged, and the leukocytes only slightly increased. The differential blood count showed a tendency toward an increased number of large mononuclear cells. The typhoid vaccine used for injection was an attenuated washed suspension of a twenty-four hour agar culture standardized to 5 billion bacteria per cc. In the first series of experiments a single dose of 1 cc. was injected intravenously, and the agglutinin titre examined after a week. However, many

[1] Gay, F. P., and Clark, A. R., *J. Am. Med. Assn.*, 1924, lxxxiii, 1296. Isaacs, M. L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 185. Jungeblut, C. W., and Berlott, J. A., *J. Exp. Med.*, 1926, xliii, 613. Gay, F. P., *Arch. Path.*, 1926, i, 590.

of the rabbits did not survive this dose; and other methods of immunization, such as a preliminary subcutaneous dose or small gradually increasing intravenous doses, were given. Fatalities also occurred when these methods of immunization were used.

Forty-one rabbits were used in these experiments; eight normal controls, seven glucose controls used to rule out the possible effect of the sugar in the colloidal iron, and twenty-five injected with iron oxide. Agglutinin titres were obtained on twenty-six of the rabbits. Eight normal controls, four glucose controls, and fifteen injected with iron.

The agglutinin titres of the normal rabbits were as follows: one, with a titre of 1:80; three with 1:640; one with 1:1280; and three with 1:2560. The titres of the glucose rabbits were as follows: two with a titre of 1:160, and two with 1:640. The titres of the rabbits injected with iron were as follows: one with a titre of 1:20; one with 1:80; one with 1:160, three with 1:320; two with 1:640; two with 1:1280, and four with 1:2560. The typhoid agglutinin formation was apparently not inhibited in any degree by the physical blocking of the reticulo-endothelial system.

All the rabbits that had received iron were autopsied, whether they died accidentally or were killed at the end of the experiment. Microscopically the large cells of the reticulo-endothelium were loaded with iron. There was little difference in the microscopic appearance of the tissues of the rabbits receiving nine injections and of those receiving nineteen injections. The continuation of the injection of iron during immunization had no perceptible effect on agglutinin formation. When the agglutinin titre dropped in rabbits that had preliminary iron injections, the titre could be restimulated by another injection of iron. The different methods of immunization elicited the same response in the normal controls, in the glucose controls, and in the iron oxide rabbits.

Conclusion: The blockade of the reticulo-endothelial system of rabbits with saccharated iron oxide had no apparent effect on typhoid agglutinin formation.

3155

Germinal epithelium in X-rayed testes of rats.

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Testes of rats have been exposed to graded doses of X-ray (determined physically and biologically) and studied over periods of time ranging from one day to six months. Doses up to ten erythema have been given at one time. The extent of degeneration produced in the seminiferous epithelium depends roughly upon amount of radiation. However, the degree of degeneration for a given dosage after a given number of days is not constant. Actively dividing cells, spermatocytes and then spermatogonia are the most easily injured. Spermatozoa disappear for a short period of time about ten days after exposure. Their absence marks the time required for conversion of spermatogonia into spermatocytes, these to spermatids, and the latter to spermatogonia.

Doses exceeding 3 erythema cause aspermatogenesis with degeneration of all seminiferous epithelium excepting so called "Sertoli" cells. In all experiments with exposure up to seven and one half erythema these cells have persisted. Doses of eight erythema and over have resulted in death. The persisting cells gradually change in structure and from them are produced spermatogonia. Cell types intergrading between these so called "Sertoli" cells of degenerated testes, which are in realtiy *Indifferent cells,* and spermatogonia have been found in these experiments. Regeneration takes place from these persisting Indifferent cells. They are, therefore, essentially germ cells. Within one month considerable recovery has taken place and in three or four months the testes are again normal. The time required for regeneration following extensive injury is about three months.

Since germinal epithelium has not been completely destroyed by radiation, as evidenced by cell division and regeneration, it cannot be said that this tissue does not take part in producing the sex hormone in X-rayed animals.

3156

Further observations on "rough" and "smooth" strains of bacteria.

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In a previous paper[1] it has been shown that single-cell strains of paratyphoid bacilli of the R and S types can be converted one into the other by suitable treatment. The S type so obtained is more stable in salt solution and more virulent than the corresponding R type, and also possesses the easily recognizable character of producing smooth, projecting colonies on an agar plate.

Rabbits have been inoculated with single-cell strains and the serum tested for agglutinating qualities. The following results have been obtained. With a single-cell smooth strain of the Schottmüller type (209 S) the serum agglutinates the homologous organism in about 1:500 dilution with a heavy flocculent precipitate. It also agglutinates other Schottmüller strains in the same manner in similar dilution. Its action on rough strains is not quite the same. Although a smooth strain serum, it agglutinates all rough strains of the Schottmüller type in higher dilution than it does the corresponding smooth strains. The precipitate, however, is finely granular instead of flocculent. It also agglutinates at least some smooth strains of the Aertrycke type in nearly as high dilution as the Schottmüller strains, *but the precipitate is always granular.*

The serum obtained with a single-cell rough strain (209 R) behaves very differently. In no instance has a "rough" serum agglutinated a smooth strain of its own group. All R strains tested, whether Schottmüller or Aertrycke type, are agglutinated by the R serum, usually in about the same dilution and always with the formation of a granular precipitate. A flocculent precipitate was never produced.

In two experiments S cultures derived from single-cell R strains by the method previously described[1] have been used. Both are of the Aertrycke type (223 and 239). The results are as follows:

[1] Jordan, E. O., *J. Am. Med. Assn.*, 1926, xxcvi, 177.

	Aertryeke Type				Schottmüller Type	
	223 S	223 R	239 S	239 R	210 S	210 R
209 S serum (Schottmüller)	+Fl	+gr	+gr	+gr	+Fl	+gr
209 R serum	0	+gr	+gr	+gr	0	+gr

In one instance, therefore, the alien R → S strain behaves exactly like the homologous S strain, in the other like the generality of R strains.

3157

The effect of double vagotomy and tracheotomy on the susceptibility of rabbits to cocaine poisoning.

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[*From the Laboratory of Pharmacology of the University of Chicago, Chicago, Ill.*]

Acute cocaine poisoning in the rabbit causes significant respiratory derangements in such a way as to lead to death of the animal. Since artificial respiration constitutes a very satisfactory method of treatment in this animal and since Richet[1] and Feinberg[2] found independently that decortication as well as decerebration raised the minimal lethal dosage of cocaine, it was thought that possibly other parts of the nervous system might contribute to the harmful effects of cocaine. This report, therefore, involves a study of the possibility of vagus involvement.

Rabbits were anesthetized with ether, double vagotomy and tracheotomy performed. After recovery from the anesthetic the animals were poisoned by subcutaneous injections of cocaine hydrochloride. It was found that this procedure raised the average minimal fatal dose from 100 to 125 mg. per kilogram in the intact animal to approximately 175 mg. per kilogram in the operated animal. Tracheotomy alone was sufficient to be equally

[1] Richet, C., *Arch. Int. Pharmacol.*, 1898, iv, 299.

[2] Feinberg, I., *Berl. Klin. wachnschr.*, 1887, xxiv, 166.

efficacious, whereas vagotomy alone was without effect in modifying the average minimal fatal dose.

These facts are interpreted to mean that in the intact rabbit, respiratory embarrassment must exist, either through spasm of the glottis or other obstruction, in such a way that the asphyxial element thereby induced very markedly contributed to the central injury produced by cocaine.

3158

Sulphur metabolism in yeast.

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The studies of Swoboda[1] in this laboratory indicate that cystin added to certain media stimulates yeast growth. This suggested a more intensive study on the actual utilization of various forms of sulphur in yeast growth.

The forms of sulphur studied were sulphate, sulphide, cystine, cystein, cysteinic acid, taurine and taurocholic acid. These were added to the usual synthetic medium as employed by Williams,[2] Miller,[3] and Swoboda,[1] with and without the "biose vitamine" and without the usual sulphate content. The yield of yeast was determined by weighing the yeast obtained after a growth period of eighteen hours. The saccharose and asparagin used in the media were purified to remove sulphur containing substances as completely as possible.

The results obtained were as follows:

1. Inorganic sulphate is the best form of sulphur for yeast growth, especially if magnesium is present with an ample amount of the biose vitamine. Neither magnesium nor sulphate could be substituted for the biose vitamine.

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[1] Swoboda, F. K., *J. Biol. Chem.*, 1922, lii, 91.

[2] Williams, R. J., *J. Biol. Chem.*, 1920, xlii, 259.

[3] Miller, Elizabeth M., *J. Biol. Chem.*, 1921, xlviii, 329.

2. Sulphide sulphur added as hydrogen sulphide in concentrations of 0.0022 mg. per 125 cc. may be used as a sulphur source by growing yeast. In higher concentrations it retards yeast growth, especially when a good sulphate containing medium is employed.

3. Cystine stimulates yeast growth slightly in concentrations of 1 to 4 mg. per 125 cc. medium. In higher concentrations and especially in a good sulphate containing medium it retards yeast growth. Cysteine behaves similarly. Possibly the harmful effect of these is in part due to their partial conversion into hydrogen sulphide.

4. Cystine sulphur is partly utilized in synthesizing new yeast protein, but a part of it also remains in the medium as sulphate.

5. In otherwise sulphur free media cysteinic acid stimulates yeast growth very slightly, but in a sulphate containing medium it retards the same.

6. Taurine in concentrations of 2 to 20 milligrams per 125 cc. has no appreciable effect on yeast growth.

7. Taurocholic acid in concentrations of 1 mg. or more per 125 cc. was toxic even in sulphate media.

3159

Specific absorption studies upon calf rennin.

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The question of the identity of pepsin and rennin has been under consideration for many years, Pekelharing,[1] Pawlow[2] and others, holding to the view that pepsin and rennin activities were not distinct, with Hammarsten,[3] and more recently Fenger,[4] claim that the two activities are different in character.

[1] Pekelharing, C. A., *Z. Physiol. Chem.*, 1896, xxii, 233-44.

[2] Pawlow, J. P., *Z. Physiol. Chem.*, 1904, xlii, 415; Schmidt-Nielsen, S., *Z. Physiol. Chem.*, 1906, xlviii, 92.

[3] Hammarsten, O., *Z. Physiol. Chem.*, 1911, lxxiv, 142-68; *Ibid.*, 1918, cii, 33-77.

[4] Fenger, F., *J. Am. Chem. Soc.*, 1923, 249-55.

The study made in this laboratory was carried on in order to help bring further proof as to the identity of these two activities.

A very active preparation of rennin was made by the method of Fenger.[4] The dried product from this method of preparation showed on the average a milk-curdling power of 1:1,000,000 in ten minutes on fresh pasteurized milk at 40° C. Reprecipitation of this material gave a final product 41.66 times as active as Armour's commercial rennin. It was more active than could be consistently prepared by the sodium chloride salting out process and it had the advantage of being free from absorbed sodium chloride. The product can be kept for a long time without losing activity.

Studies were then carried out in order to determine if a more active product could be prepared. This was attempted by absorbing the rennin activity from the calf preparation, on solid purified casein suspended in solutions at various pH values. In these studies it was observed that the rennin activity could be absorbed to the extent of 90 to 95 per cent when the pH value of the solution was from 4.4 to 4.6. This was shown to be true by testing the filtrates, after absorption, for milk curdling power. It was also observed that the absorbed rennin activity could be recovered from the casein to the extent of 75 per cent to 80 per cent by bringing the solution to a pH value of from 6.4 to 6.6 by means of $NaHCO_3$. The recovered rennin, however, contained considerable casein, which prevented the exact determination of the purity thereof, hence it was thought that another protein, of different solubilities than casein, might be used to advantage.

Dry, powdered, coagulated, acid treated egg white was then used as the absorbing medium and it was found that only from 17 per cent to 20 per cent of the rennin activity was absorbed by the egg white. It was known from work being carried on in the laboratory by Dr. T. L. McMeekin that hog's pepsin could be absorbed practically quantitatively on coagulated egg white. Studies were then carried out by the method of McMeekin[5] to determine if the absorbed material on the egg white possessed peptic activity. It was found that solutions of the calf rennin

[4] Fenger, F., *J. Am. Chem. Soc.*, 1923, xlv, 249-55.

[5] McMeekin, T. L., Doctorate Dissertation, U. of Chicago, 1925, "Studies on the Purification of Pepsin."

preparation when incubated with egg white in 0.3 per cent HCl for two hours at 40° C. showed digestive powers from 36 per cent to 37 per cent of that of 1:3,000 hog pepsin solution of the same per cent strength. These experiments also showed that this pepsin or pepsin-like material contained in the calf rennin preparation can be removed from its solution to the extent of 84 per cent to 92 per cent by absorption on egg white without appreciable removal or destruction of the rennin activity. The filtrates resulting from the egg white absorption process possessed only slight pepsin proteolytic power, but had from 87.5 per cent to 91 per cent of the milk curdling power of the original rennin solutions.

It was also observed that a mixture of hog preparation and calf preparation when incubated with egg white in 0.3 per cent HCl showed digestive powers equal to the sum of the two. The study shows, therefore, that practically complete separation of hog pepsin and calf rennin can be made by absorption on acid treated egg white and that the calf preparation contains two activities which can be separated from each other without destruction of either one, by absorbing the respective activities upon the proper proteins at properly controlled pH values.

3160

Effect of visual impulses on the posture of the head.

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It is a well known fact that the normal position of the head is maintained through impulses coming mainly from the otoliths (labyrinthine righting reflexes on the head). An animal with both labyrinths destroyed will not be able to preserve the normal posture of the head when held in the air in various abnormal body positions. According to Magnus,[1] the cat, the dog, and the monkey are exceptions to this rule, in that through visual im-

[1] Magnus, R., *Koerperstellung*, Berlin, 1924, p. 19, 224, 261, 267.

pulses they are still capable of righting their head even after bilateral labyrinthectomy.

In our experiments we have employed normal puppies, rabbits, guinea pigs, fowls and pigeons. All these animals are capable of maintaining the normal posture of the head, as indicated by the direction of the snout or beak, when held in the air in the side position or in the supine position. The latter involves a very sharp ventroflexion of the head, the snout or beak being turned towards the tail. With one eye kept closed by pasting a strip of adhesive tape over it, all these animals are capable of preserving the normal posture of the head when held in the air in the lateral position in such a fashion that the open eye is uppermost. If, however, the closed eye is uppermost, the head is held in the normal position only for a few seconds and then gradually begins to sink sideways, until it assumes a position characteristic of an animal without labyrinths. The difference in the two lateral positions is especially striking in the case of the fowl.

When the animal is completely blindfolded (by pasting adhesive tape on both eyes) it loses completely the symmetrical righting reflexes on the head when held in the air in the supine position. Here, too, the posture of the head is normal for a short while, but this posture is not maintained, as the head gradually begins to sink, until it hangs lifelessly with the snout or beak directed downward. When the animal is in this condition, removal of the plasters from the eyes causes an immediate righting of the head.

From these results it is evident that while the otoliths are necessary for the elicitation of the righting reflex on the head, when an animal is held in the air in the supine position, the impulses coming from them are not sufficient for maintaining the normal position of the head under these conditions. The visual impulses play an important rôle in keeping the head in the normal position once it is brought there through labyrinthine impulses.

3161

Experimental control of polarity in corymorpha palma.

C. M. CHILD.

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Corymorpha palma is a large, unbranched tubularian hydroid abundant on the Southern California coast. Many different lines of evidence show that in this form, as in others, the physiological axis is primarily represented by a quantitative differential, a gradient, in metabolic activity and associated conditions in the protoplasm. From the most active or 'high' end of this gradient the apical structure, the hydranth, develops and from lower levels the stem and base.

The forms resulting from the reconstitution of isolated pieces of the stem of Corymorpha differ with length of piece, level of body, physiological age and condition of animal, etc., and also with differences in various external conditions. The forms produced fall into three chief groups: (1) uniaxiate, a single individual or an apical part, which may develop from either distal or proximal end of the piece, or under certain conditions, from the side; (2) biaxiate, with an apical end developing from each cut surface and other parts in order from each apical end, as far as the length of the piece permits; (3) intermediate forms, biaxiate as regards the apical ends, but with one or more proximal or basal regions developing from the side of the stem between the two apical regions. In lots of similar pieces from similar stems the frequency of uniaxiate and intermediate forms is much higher in pieces allowed to lie undisturbed on the bottom of the container than in pieces supported above the bottom on loose absorbent cotton. The frequency of uniaxiate and intermediate forms is higher in pieces undisturbed on the bottom than in pieces moved about and turned over every few hours. Pieces above a certain length lying on the bottom and subjected to the action of depressing agents, *e. g.*, alcohol, LiCl, chloretone, ethyl urethane, give a higher frequency of uniaxiate and intermediate forms and in such forms a higher frequency of large proximal parts and multiple basal ends, than pieces in well aerated water.

The factors concerned in determining new polarities in such

pieces are: first, the proximal cut surface which establishes a region of high metabolism and a new gradient opposite in direction to the original in biaxiate forms; second, the differential exposure of the ends or regions of pieces lying on the bottom. All the evidence indicates that this differential exposure is not a matter of contact and lack of contact with a solid surface, but rather of the more rapid respiration possible on the free surface than on that in contact. After a certain period of such differential exposure the free surface becomes the high end of a gradient and develops into an apical end, while the surface in contact becomes the low end and develops into a proximal body level or a basal end. In the presence of depressing agents the development of basal parts from the region in contact is further favored by the decreased motor activity in consequence of which a particular region remains more continuously in contact, and by the differential inhibiting action of the agent, which also favors the increase in size and number of basal parts.

The following table gives in percentages some of the data obtained:

	Length of pieces	No. of pieces	Conditions	Uniaxiate and intermediate	Biaxiate	Dead
I	1/5 naked region	100	On cotton	24	73	3
		100	On bottom	72	20	8
II	1/10 naked region	50	Moved and turned	60	36	4
		50	Undisturbed	96	4	
III	1/4 naked region	20	Control in sea water	35	65	
		20	Alcohol 2% 48 hrs.	75	25	
IV	1/2 naked region	20	Control in sea water	25	75	
		20	LiCl m/20 24 hrs.	65	25	10
V	1/5 naked region	40	Control in sea water	47.5	52.5	
		20	Chloretone 1/5000 24 hrs.	85		15
		20	Ethyl urethane m/250 24 hrs.	80	10	10

3162

Antiperistalsis in the upper third of the esophagus in man.

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The subject of this investigation was a child of thirteen years who, at the age of four, swallowed strong acids, and in consequence developed almost complete cicatricial stenosis of the esophagus, the upper end of the occlusion being about 5 cm. below the level of the sternum. During the nine years the child had taken nourishment almost exclusively by a gastrostomy tube. For months at a stretch not even a drop of water could pass through the esophagus into the stomach. Occasionally an opening of 2 to 3 mm. diameter appeared, allowing water and milk to pass from the mouth into the stomach. Evidently a chronic spasm of the injured portion of the esophagus had developed on the top of the mechanical stricture. During an investigation of possible means of controlling the esophageal spasm, the child frequently swallowed small quantities of barium milk while fluoroscopic observations were being made. There was little or no permanent dilation of the end of the esophagus above the occlusion. The presence of the barium milk in this region led at once to vigorous movements of the esophagus resembling the movements of the small intestine above an obstruction, as originally described by Cannon.[1] One could make out local rings of constriction (segmentation movements) at different levels, as well as regular peristalsis. There frequently appeared vigorous antiperistalsis, forcing some of the barium milk towards and even into the mouth. The esophagus was able to empty itself completely in this way. We could secure no evidence that the mechanism was under voluntary control. The antiperistalsis did not induce any special sensation, such as nausea. Material in the upper end of the esophagus was felt as "something stuck in the throat," whether or not antiperistalsis was present.

The musculature of the upper third of the esophagus in man is striated, with motor control via the vagi nerves. There is no

[1] Cannon, W. B., "Mechanical Factors of Digestion," 1911, p. 131.

evidence that local automatism ever develops in this part of the esophagus, at least as long as the vagi are intact. We have, therefore, in this case an illustration of antiperistalsis coordinated reflexly through the central nervous system.

3163

In vitro studies on ammonia and urea formation by tissues.

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This investigation was undertaken in the hope that light would be thrown on the subject of desaminase action by the various tissues of the body. The subject is in a more or less unsettled state, some workers[1] even disclaiming a true deaminizing action in tissues, believing that the ammonia production is due to deamidase action. Still others[2] take the view that the amino acids instead of yielding ammonia, are attacked in the carbon chain itself, thus being broken down and oxidized, the products formed producing cyanic acid which can be converted into urea by the addition of ammonia formed by deamidase action.

In this work an attempt was made to study the action of ammonium salts, or of mixtures of amino acids and peptides, or of amino acids alone upon ammonia and urea formation or utilization by tissues *in vitro*.

The tissues used were obtained from recently killed dogs or from the abattoir, in this case using hog tissue. These were minced as soon as possible, mixed with a buffer phosphate solution and after an hour the juice was filtered and pressed out. Of this well mixed fluid, 25 cc. portions were taken and incubated with the additions referred to. All the necessary control estimations were made and the methods used were critically studied before using them on the problem. The tissues studied were liver and kidney.

[1] Luck, J. M., *Biochem. J.*, 1924, viii, 814.

[2] Weiner, E. A., "Chemistry of Urea," 1923, London.

The main observations made are as follows:

1. Ammonium salts added to the tissue extracts inhibit ammonia production. The chloride is most inhibiting. The lowered ammonia production is usually accompanied by increased urea production. Thus, in four experiments ammonium as acetate, phosphate or lactate stimulated urea production, while in two experiments all the ammonium salts retarded urea formation, the phosphate being the least inhibiting.

2. Half saturation of liver tissue extract with carbon dioxide stimulates urea production.

3. Addition of trypsin hydrolyzed casein to the tissue extract caused a marked increase in both ammonia and urea after incubation. The ammonia formed may come from either amide or amino groups in this case.

4. Acid hydrolyzed casein was also employed as a substrate free from amide nitrogen. When this was added to the tissue extract distinct increase in both ammonia and urea formation was observed with liver tissue. The ammonia formed suggests deaminase action but may possibly be due to a stimulating or activating action of the amino acids in the deamidase tissue extract. With kidney the ammonia is increased but urea is lost or destroyed.

5. Addition of large amounts of ammonium salts to liver extracts followed by immediate heat coagulation is accompanied by a loss of 82 to 87 per cent of the ammonia added. The ammonia is in part converted into urea and the remainder changed otherwise.

6. Addition of either small or large amounts of ammonium salts to kidney extract is accompanied, either in heat coagulation or during incubation, by a loss in urea and an increase in ammonia.

3164

Conditions under which subcutaneously injected epinephrine gives a hemodynamic effect.

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The statements in the literature regarding the hemodynamic effects of epinephrine injected hypodermically are few and conflicting. Some investigators[1] report feeble pressor effects; others[2] are as certain that there is no effect unless perchance epinephrine is injected quite directly and accidentally into a small venule. Even those who report feeble positive effects (Meltzer and Auer) were probably mistaken in the facts and interpretation, as we will point out more specifically in our detailed report.

In the course of some work in which epinephrine was administered subcutaneously in dogs under light paraldehyde (and morphine) anesthesia an appreciable rise of blood pressure was noted during the 3 to 4 minutes following the injection. Repetition of the experiments yielded questionable results; for there was either no immediate rise in blood pressure following such injection or the progressive rise in blood pressure could be readily explained on the basis of recovery from the light paraldehyde anesthesia of the animals. However, it was soon noticed that gentle massage of the injected area effected an appreciable hemodynamic effect and this led to a systematic investigation of the phenomenon.

Four to twelve kg. dogs were used for the most part under light paraldehyde anesthesia (1 to 1.5 cc. per kg.) reinforced as found necessary by subcutaneous injections of morphine sulphate (¼ to ¾ gr.). Blood pressure records were taken from the carotid artery. Hypodermic injections of epinephrine were made

[1] Meltzer and Auer, *J. Exp. Med.*, 1905, vii, 59; *Zeit. f. Phys.*, 1905, xviii, 689. Foerster and Benkovics, *Zeit. f. ges. exp. Med.*, 1926, xlix, 1. Thiess, cited by Braun, Local Anesthesia, Lea & Febiger, 1914, 144. Goetsch, *Pa. Med. J.*, 1920, xxiii, 431, and others by same author between 1920 and 1922. Lyon, *J. Exp. Med.*, 1923, xxxviii, 655.

[2] Patta, *Archiv. di Farmac.*, 1905, iv, 329. Janeway, The Clinical Study of Blood Pressure, Appleton & Co., 1907, p. 223. Biedl, Innere Sekretion, Urban und Schwarzenberg, Wien and Berlin, 1913.

in a great many regions of the body in doses of ½ to 6 cc. (Parke Davis & Co.) in original dilution or more or less diluted with distilled water. In most instances the area so injected was gently or vigorously massaged immediately after injection and at various time intervals following injection. In a few instances the animals were injected subcutaneously from 12 to 19 hours prior to the massage.

Massage of areas injected with epinephrine was followed by a typical rise in blood pressure (15 to 180 mm. Hg.) secured with great regularity 15 to 17 seconds after the beginning of massage. The rise and fall of blood pressure are either abrupt and evanescent; or the fall may be gradual—the return to normal may take occasionally 10 to 15 minutes. In fact, the rise in pressure may be so protracted that the curve simulates the effect commonly seen after the first injection of pituitrin. The magnitude of the pressor response (with or without typical vagus action of medullary origin) depends in part on the dose of adrenalin injected but more particularly on:

1. *Time following injection.* Early after injection the response is likely to be poor or entirely absent, due to the local vasoconstriction of the injected area; for the effect elicited from a given area becomes more pronounced when the original intense vaso-constriction gives place to a dilatation of the subcutaneous vessels (secondary paralysis) or to hemorrhages into the necrosis of the skin (moist gangrene). Furthermore, by injecting small doses of $Na\,NO_2$ subcutaneously into the area into which the adrenalin is subsequently injected, local vasoconstriction is prevented and the rise of pressure on massage is immediate.

2. *The Type and Depth of the Anesthetic Used.* The lighter the anesthetic the more certain and the more pronounced is the response. Under deep morphine sulphate analgesia reinforced by ether for the purpose solely of preparing the animal for registration of the blood pressure, the response comes on very early and is quite pronounced. Paraldehyde anesthesia is the most serviceable because of the lightness of the anesthetic state and was most commonly used. Positive results were never obtained under barbital anesthesia (Barbital Sodium). The hemodynamic effect was also absent under paraldehyde in case this anesthetic was administered in doses which were excessive. The effect of massage was not due to a pressor response as a result of stimulation of

the sensory nerves made hyperexcitable by the subcutaneous injection of the adrenalin for (a) the reaction was delayed some 17 seconds, the rise was abrupt, and (b) stimulation of similar control area gives uniformly a depressor effect. The typical pronounced effect following massage could furthermore be abolished either by administration of ether (inhalation) or by the intravenous injection of small quantities of paraldehyde or barbital-sodium.

3. *The Fatigue or the Depression of the Peripheral End Organs.* (a) It was found early that the oft repeated massage of a given area is followed by a progressively weaker response. After ½ hour or more of rest massage of this area gave again the marked response. This fact can be variously interpreted. We have interpreted it provisionally as possibly indicating fatigue or depression of the peripheral end organs; for

(b) The pressor effect of intravenously injected epinephrine solution can be materially diminished by the intravenous injection of small amounts of ether.

There is no question that massage of a cutaneous area previously injected with epinephrine gives a pressor effect as a result of epinephrine reaching the general circulation, for (a) the effect can be abolished or reversed by the intravenous injection of ergotamine tartrate (1 mg. per kg.); (b) there is some indication, perhaps, that the response can be intensified by the intravenous injection of a weak cocaine-hydrochloride solution and; (c) a saline extract of the subcutaneous tissue reveals on intravenous injection appreciable quantities of epinephrine as long as previous massage of this area was followed by a pronounced pressor effect.

It is a surprising fact that a typical and pronounced pressor effect as a result of massage could be elicited as late as 19 hours after the subcutaneous injection of 1 cc. of epinephrine and that a saline solution extract of this subcutaneous tissue possessed marked pressor effect on intravenous injection.

The importance of these findings and their application in the therapeutic use and diagnostic value of epinephrine (Goetsch test) are more or less obvious but will be discussed in the detailed report.

3165

Calcium absorption.

OLAF BERGEIM.

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If a definite amount of an unabsorbable compound, such as ferric oxide, be mixed with a diet and the feces be analyzed for iron and for any food constituent, such as calcium, it is possible to determine the percentage absorption or "utilization" of the latter without an accurate separation of the feces. If the ratio of calcium to iron in the food be 10:1 and in the feces 5:1, calcium unabsorbed amounts to 50 per cent and calcium abserbed would of course also be 50 per cent.

Using this method, it has been shown that lactose in contrast with other common sugars, markedly increases the absorption of calcium. Lower fatty acids have a more favorable effect than higher fatty acids. Antirachitic substance produces the expected result.

In the same way, if animals fed an iron containing diet are killed at the height of digestion and analyses made of the intestinal contents at different levels, the percentage absorption of food products in different parts of the intestines may be estimated.

Experiments of this type on rachitic albino rats on diets with or without cod liver oil give interesting results. Using a calcium-high phosphorus-low diet rats with or without cod liver oil show considerable calcium absorption in the upper small intestine. This type of rickets is not due, therefore, to a failure of calcium absorption. Excretion of absorbed calcium into the lower bowel does, however, bring about a negative or subnormal calcium balance.

Phosphorus, on the other hand, is actually excreted into the upper intestines. Animals given cod liver oil make up for this loss by absorption from the cecum and large intestines. Rachitic animals do not and, therefore, the phosphorus balance remains negative.

On a milk diet, high in both calcium and phosphorus, both are absorbed to a considerable degree in the small intestine. It is in

the lower bowel that the balance is swung one way or the other depending primarily on the ability of the tissues to utilize these elements.

3166

Botulinum toxin in the alimentary tract.

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The great variation in the susceptibility of different animal species to oral injections of botulinum toxin may conceivably be due either (1) to the destruction or adsorption of the toxin by the intestinal contents, or (2) to the difference in permeability of the intestine to the toxin. Type A botulinum toxin was used in these experiments.

There was no evidence that botulinum toxin was adsorbed by the intestinal contents of guinea pigs *in vitro*, even where the pH was shifted with M/3000 HCl or Na_2HPO_4.

A loop of small intestine in each of six rabbits was injected with botulinum toxin and perfused with blood from the same animal for intervals varying from 30 minutes to 2 hours and 20 minutes. Very small quantities of toxin were demonstrated in the perfused blood, often only sufficient to produce symptoms of botulism in mice receiving 0.5 cc. quantities. No toxin was ever demonstrated in 0.1 cc.

In each of two hogs a loop or small intestine was perfused in a similar manner. In one case a very small amount of toxin was found in the blood, a mouse receiving 0.5 cc. of serum taken after an hour and a half of perfusion died in 4 days. In the other case toxin was not demonstrated. The toxin introduced into the ligated loop of intestine showed little if any decrease in potency during the course of the perfusion experiments.

Hogs were found to be very resistant to large oral doses of toxin; in some cases as much as ten million M. L. D.'s for mice were fed without producing any ill effects. Toxin was not demon-

strated in one cc. of blood taken after 90 minutes from a hog which had been fed nine million (mouse) M. L. D.'s.

Toxin was not demonstrated in the blood of rabbits 90 minutes after feeding botulinum toxin. Two rabbits received intravenous injections of small graded doses of toxin. Toxin was demonstrated in the blood stream 18 hours later.

Two rabbits receiving large doses of toxin in the small intestine showed symptoms of botulism within seven days. One died on the seventh day and the other on the fourteenth day. Toxin was not demonstrated in a half cc. of blood taken from each of these animals after 90 minutes or in one cc. taken after 18 hours.

Two rabbits received intracecal injections of toxin. One of the rabbits remained normal. The other died the following day and toxin was found in the blood.

Toxin could not be demonstrated after 90 minutes in the blood of guinea pigs which had received injections of toxin in the ligated stomach but was demonstrated in the serum from the heart blood taken after death. Toxin was not demonstrated in the blood of animals receiving injections of toxin in the ligated small intestines or ligated cecum, either after 90 minutes or in the serum from the heart blood taken after death. There was one exception in a guinea pig which had received an intracecal injection, the cecum of this animal being ruptured.

Two guinea pigs were given intracecal injections of toxin; one died seventeen days later with typical symptoms of botulism; the other remained normal.

Mice fed botulinum toxin failed to develop any symptoms of botulism. Toxin was demonstrated in the stomach and small intestines of mice 3 hours after they had been fed toxin but not 12 hours later.

When susceptible animals are fed toxin the evidence from these experiments indicates (1) that there is some absorption from the small intestine and (2) that there may be some continued absorption from the cecum. Further studies are being made on the absorption of toxin from the cecum.

3167

A seasonal variation in the excretion of phenols.

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By total urinary phenols we mean substances which react with the reagent in the Folin and Denis colorimetric method. The subject studied in this experiment abstained from meat, fish, coffee, tea, alcohol and tobacco; an average figure was taken for a four day sample during such weeks in which there was no sickness, accident or vacation.

Although the level for 1923 is higher than that for 1924, yet, within each year we find about 100 mg. more phenols excreted per day during the summer months of June, July and August, than during the winter—meaning December, January and February; the spring and autumn show intermediary figures, and those for the autumn would probably have been higher had not September been vacation-month. If we combine the two years of 1923 and 1924, we find the following:

Spring, 60 days, average 411 mg.;
Summer, 71 days, average 463 mg.;
Autumn, 60 days, average 388 mg.; and, finally,
Winter, 84 days, average 345 mg.; or for 3 winters,
124 days, average 353 mg. of phenols.

As to the cause of such a seasonal variation we at first thought that the analyses were perhaps carried out at somewhat higher temperatures during the summer and that this might be a factor. Tests showed that a standard set at 20° Centigrade compared with a similar standard set at 30° Centigrade gives an increased color in the latter equivalent to a 20 per cent error; however, a certain sample gave 333 mg. of phenols at 30° Centigrade and 338 at 20° when compared with the same standard at the corresponding temperatures. We always kept the standard and the unknown at the same temperature, so this error was not introduced.

Secondly, because the phenols in an exaggerated manner followed the Nitrogen fluctuations of 1924-25, we have included the records of the winter 1925-26, which demonstrates that Nitrogen

is not the main factor; for with 12.66 g of N per day for the winter against 11.75 for the summer, the phenols remain low. Likewise, nothing definite may be concluded from the urinary volumes, or from the number of defecations per day, which latter were almost the same per season.

Furthermore, we have no direct evidence that with the diet employed one ingests more phenol-forming bacteria during the summer than the winter, nor that the pH of the intestine differs markedly with the season.

Finally, we must consider the seasonal difference in the intensity of sunlight. Koch and Reed[1] found that in about two-thirds of the dogs which they studied, an exposure to ultra-violet light gave higher figures for substances of probable phenol character in the blood, which substances reacted with the uric acid reagents of Benedict or of Folin. Also, since tyrosin is acted upon by sunlight to give melanins, and by bacteria to give phenols, it may be worth while to investigate further if the extra sunlight has to do with the higher phenol excretion during the summer.

3168

Influence of some salts which change P. D. on the phagocytosis of pneumococci.

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It has long been known that virulent strains of pneumococci or other bacteria are not readily ingested by leucocytes and that avirulent strains are generally ingested readily. It has also been established that the presence of acids, alkalies, salts and other reagents modify the velocity or the extensiveness of phagocytic reactions.

In studies on the parallel relations between virulence, electrophoretic potential difference (P. D.), agglutinability and other characteristics of bacteria, strains of pneumococci of different

[1] Koch and Reed, *A. J. Physiol.*, 1926, lxxv, 351.

virulence for white mice were examined in considerable detail.[1] The pneumococci designated A are virulent Type I organisms. The strains B and C are variants of A which were derived by Blake and Trask by growing strain A in the presence of specific anti-serum. The minimum fatal doses for white mice are: 0.5×10^{-7} cc. for A; 0.5×10^{-3} cc. for B; and 0.5+ cc. for C. The P. D. is highest on A and lowest on C.

The experiments reported here were undertaken to determine the extent to which A, B and C strains of pneumococci are taken up by phagocytes under conditions which were designed to decrease or increase the P. D. It has been established[1] that the virulence for white mice is altered in a parallel manner when the P. D. is increased or decreased.

It has been found that in the presence of normal rabbit serum or of immune horse serum, the sequence of decreasing phagocytic indices is: *C, B, A.* For the opsonic indices, the series is: $A = B > C$. The enhancement of phagocytosis by the presence of unheated or heated immune serum is approximately proportional to the concentration of the serum.

It has been found[1] that the sequence of the relative magnitudes of the P. D. values for the A, B and C strains is inverted by frequent washings with water from $A > B > C$ to $C > B > A$. It was found, correspondingly, that the sequence of the phagocytic indices was also inverted.

If the P. D. on the pneumococci be reduced by such a salt as lanthanum nitrate, the phagocytosis is increased proportionately. If the P. D. be increased by sodium oleate,[2] the phagocytosis is proportionately reduced. The phagocytic indices were similarly modified successively when suspensions of the pneumococci were treated with lanthanum nitrate, washed, and then with sodium oleate, and when the procedure was reversed.

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[1] Falk, I. S., Gussin, H. A., and Jacobson, M. A., *J. Inf. Dis.*, 1925, xxxvii, 481; Falk, I. S., Jacobson, M. A., and Gussin, H. A., *ibid.*, 495, 499; Falk, I. S., and Jacobson, M. A., *ibid,* 507; 1926, xxxviii, 182, 188.

[2] *Cf.*, Falk, I. S., and Yang, S. Y., *J. Inf. Dis.*, 1926, xxxviii, 1, 8.

3169

Electrophoretic potential and virulence of diphtheria bacilli.

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Measurements of electrophoretic velocities on strains of pneumococci and other organisms indicate that many vital phenomena are closely associated with the electrokinetic P. D. between the organism and its menstruum.

It has become evident from the work of Falk and his associates[1] that there are significant parallelisms, direct or inverse, between electrophoretic potential, virulence, agglutinability and other characteristics of bacteria. For pneumococci of the types 1, 2, 3, 4 it has been found that the P. D. is higher the greater the virulence for white mice and vice versa.

The work here reported was undertaken to determine whether or not P. D. would parallel virulence of various strains of diphtheria bacilli as well as to obtain comparable data in regard to P. D. on avirulent or pseudodiphtheria bacilli.

The cataphoretic potentials on corynebacteria were determined by the method described and used by Falk, Gussin and Jacobson.[1] For reasons discussed by them results are expressed in terms of observed velocities (μ/sec) instead of expressing the electrical P. D. at the interface between the bacterium and the menstruum in millivolts.

P. D. in observed velocities (μ/sec) may be converted into millivolts by multiplying by the factor 1.3. Ten measurements of velocity (5 with one and 5 with reversed orientation of the electrical field) were made at stationary layers V_s133, 497, in the same medium in which the bacteria were grown. The same methods were used in measuring velocities of cells in distilled water.

The electrokinetic P. D. on 65 strains of corynebacteria parallel the virulence (toxigenicity) of these bacteria. Measurements of potential on 48 hr. veal broth cultures of toxigenic strains give

[1] Falk, I. S., Gussin, H. A., and Jacobson, M. A., *J. Inf. Dis.*, 1925, xxxvii, 481, 495, 499; Falk, I. S., and Jacobson, M. A., *ibid.*, 507; xxxvii, 182, 188.

potential differences of 4.2 to 1.0 (μ/sec) with an average of 3.4 (μ/sec). When these strains are grown in veal peptone broth (48 hour cultures) the P. D. falls, giving values from 3.0 to 0.8 (μ/sec).

Measurements of cataphoretic potentials on avirulent (non-toxigenic) strains which ferment saccharose quite uniformly give very much higher values (P. D. 12.1 (μ/sec)). Non-fermenting avirulent cultures gave potential differences of 7.9 to 10.0 (μ/sec). Strains with a P. D. of intermediate values, 4.4 to 6.2 (μ/sec), are according to morphological and biochemical characteristics typical diphtheria bacilli but are not virulent. These differences are not due to changes in pH of the menstrua as the same potentials were observed in sugar free buffered broth (pH 7.2).

Distilled water suspensions of corynebacteria washed 3 times in distilled water gave the following potentials: all avirulent strains P. D. = 20.0 — 34.0 (μ/sec) with an average potential of 26.0 (μ/sec); all virulent strains range in electrophoretic velocities (P. D. μ/sec) from 2.6 to 11.3 (μ/sec) with an average P. D. of 7.0 (μ/sec).

Apparently no parallelism exists between the minimal fatal dose of washed bacilli suspended in distilled water and their P. D. in the same menstruum. The lowest values observed in broth cultures were found on the Park No. 8 strain of C. diphtheria. The degree of toxicity parallels P. D., *i. e.*, low potential is a concomitant of high toxicity. Salted out, purified toxic materials depress P. D. in a quantitative manner. The power of virulent strains

TABLE I.
Electrophoretic Potentials on Corynebacteria.

Cultures	pH average	48 hr. broth cultures (pH 7.2)			Dist'd H_2O suspensions P.D. (μ/sec) average
		P.D. (μ/sec) average	Sugar free buffered (pH 7)		
			pH average	P.D. (μ/sec) average	
Virulent (40 strains)	6.2	3.4	7.2	3.3	7.0
Non-ferm.	7.0	8.1	7.4	9.1	25.0
Aviru- Dext. ferm	5.8	5.5	7.2	8.0	31.00
lent Sucr. ferm. (25 strains)	5.8	12.0	7.2	9.0	34.00

to elaborate toxin diminishes with increases in P. D. and is enhanced when the P. D. is decreased with suitable reagents. If P. D. and M. L. D. of broth cultures are determined daily for 12 days a direct parallelism is shown to exist.

Rapid identification of virulent diphtheria bacilli can be effected by determinations of P. D., especially of distilled water suspensions prepared from 18 hour glycerin agar cultures.

3170

Influence of anti-serum and of animal passage upon virulence and electrophoresis of pneumococci.

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In earlier publications[1] we discussed at length the parallel relations between the virulence and the electrophoretic potentials of pneumococci. We reported that variant strains (Blake) of type I pneumococci, which differ in their virulence for mice, also differ in a parallel manner in their electrophoretic potentials.

In the studies reported here we have undertaken to determine whether "rough" colony varieties could be produced from the Blake Type I pneumococcus (designated as A) and its variants (designated as B and C respectively). These variants are derivatives of the A strain, and were obtained by Blake and Trask by growth in the presence of specific anti-serum. We have also undertaken a series of experiments to determine changes in virulence and P. D. upon successive passage through white mice of cultures of significantly different virulence and P. D.

We have found that the sequence of decreasing virulence for white mice, decreasing P. D. and increasing agglutinability is: A, B, C. The A, B and C strains form "smooth" colonies on peptone, serum or blood agar plates. Strains which give "rough"

[1] Falk, I. S., Gussin, H. A., and Jacobson, M. A., *J. Infect. Dis.*, 1925, xxxvii, 481; Falk, I. S., Jacobson, M. A., and Gussin, H. A., *ibid*, 495, 499; Falk, I. S., and Jacobson, M. A., *ibid.*, 507; 1926, xxxviii, 182, 188.

colonies on blood agar plates have been produced from the A, B and C cultures by growth in broth to which specific anti-serum had been added. After 23 transfers in serum broth, none of the cultures were completely converted to the rough varieties. Organisms of both the S and R varieties could be recovered. Some of the S varieties, which were recovered after 23 transfers in broth containing anti-serum, showed the virulence and the electrophoretic potential characteristic of the original cultures. Some of the S varieties show reduced virulence and potential. The rough varieties recovered after 12 transfers of B and C strains in broth plus anti-serum showed the same virulence and potential as the original B and C cultures. Hence, it appears that strains of pneumococci which differ significantly in virulence are not necessarily correspondently separable into S and R categories.

The original A strain gives a prompt precipitation reaction with specific anti-serum. The original B and C strains and the "rough" derivatives of A, B and C strains give only a slight, delayed reaction after 24 hours.

The A and B strains showed no significant changes in virulence, potential or agglutination after 4 and 8 passages through mice. Passage of the C strain through mice resulted in a reversion of its characteristics to those of the A strain.

In all cases studied, alterations in the virulence of pneumococci for white mice are accompanied by parallel alterations in electrophoretic potential and by reciprocal alterations in agglutinability.

3171

Modification of development in chick embryos induced by ultra-violet radiation.

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This is the sixth of a series of studies on radiation, four of which have concerned themselves with differential modification

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of embryonic development. The previous work includes experiments on Arbacia eggs and sperm, and on Fundulus eggs. The results in all the experiments agree in their more general features, namely, those regions which at the time of exposure have the highest rates of physiological activity, are the most readily modified in their development. Such modifications may be brought about by exposures made at various intervals after fertilization, as well as before fertilization. Experiments with hens' eggs yield the same general results, when exposed before and after incubation.

The complete spectrum of the Hg vapor arc (running at 110 v. D. C. at a distance of 10 inches) served as a source of radiation. Eggs were exposed at intervals, up to 64 hr. after incubation, as well as before incubation. Since it was found that exposures through the shell were entirely without effect, it was necessary to remove a small part of the shell and underlying membrane, and thus expose the blastoderm directly to radiation through the very thin layer of albumen which covered the surface of the yolk of the egg. Such a thin layer does not screen off the effective radiation. Exposures were from 1/5 min. to 10 min. in duration. It was found that later stages of development required less radiation to produce a given result, and would be killed with doses of 5 to 10 min. which produce only developmental modifications when exposures are made before incubation.

Before incubation, or reincubation, the piece of shell which had been removed was replaced, and the egg sealed with paraffin. Development was usually allowed to continue for three days, although some eggs were removed from the incubator after 24 or 48 hours.

Results. Among unoperated unexposed controls the percentage of normal eggs which developed to the three-day stage was from 85 to 90. Operated controls yielded about 60 per cent normality, while operated exposed eggs yielded only about 10 per cent normal embryos. These calculations are based on totals of 117, 114, and 493 eggs respectively. Exposure of operated eggs through plate glass one cm. thick produced a normality of 75 per cent, indicating that the effective radiation did not get through the glass.

The experimentally produced abnormalities are typically differential, that is to say, the regions which at the time of exposure had the highest rates of physiological activity, are most

readily modified. Accordingly, the fore-brain, particularly the anterior median region, is extremely susceptible to modification in the early stages of development. In later stages, other regions of the brain are also modifiable, particularly the hind-brain at the time of flexure and turning of the head. As the embryonic axis elongates posteriorly, the neural fold and somite region cease to develop normally in a large percentage of the cases indicating a double gradient of differences in susceptibility relations along the axis.

From these results we may conclude that there is a difference in susceptibility to modification of development by means of ultraviolet radiation along the axis of the chick embryo. The difference is in general coincident with an anteroposterior gradient in early stages, although in later stages, such a gradient is complicated by the appearance of highly susceptible regions which express local rapid development or differentiation, as for example, in the region of the hind-brain or in the posterior somite region. The fact that eggs exposed before incubation show in their later development the same axial differences in susceptibility to developmental modification as they do later, indicates the early presence of an axiate organization in the egg, even before it can be detected morphologically.

3172

The nutritional value of chlorophyll as related to hemoglobin formation.

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A study of the problem of organic precursors of the hematin part of hemoglobin shows that no attention has been directed toward the possible relation of vitamines therein, whether this be as a direct precursor, or as involved in the use of such possible precursors as chlorophyll.

The problem was to determine whether rats could be rendered anemic by the absence of one or more vitamines in a synthetic diet

and whether phaophytin, obtained from chlorophyll, functions as a hemoglobin precursor in anemic rats, however this anemia may have been brought about. Rats kept upon such diets were systematically followed as to weight, blood hemoglobin and red blood cell count. The tentative conclusions arrived at as applied to rats are the following:

1. Synthetic rations containing 18 per cent casein and deficient in Vitamines A, B and E do not produce anemia but do produce an unusually high hemoglobin content.
2. Phaophytin may not be substituted for any one of the Vitamins A, B, or E.
3. Casein at a 10 per cent level produces a lower hemoglobin content than does casein at an 18 per cent level.
4. Casein extracted with acetic acid and aerated is less efficient in maintaining hemoglobin than is casein extracted with lukewarm alcohol.
5. Casein is much more efficient in the maintenance of hemoglobin than is gluten.
6. Experimental anemia may be produced on synthetic rations using wheat gluten as the protein and this anemia may be at least partially and temporarily alleviated by the addition of 0.2 to 1 per cent phaophytin to the ration.
7. It appears that proteins contain an important precursor of hematin and that since casein contains this in greater quantity than wheat gluten, the indications are that possibly tryptophane is involved.

3173

Gonad cross-transplantation in Sebright and Leghorn fowls.

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I. INTRODUCTION.

The main problem attacked was the question of a difference in the endocrine secretions of the testes of the Sebright and the Leghorn. The Sebright male is so-called "hen-feathered" as it has a feathering similar to that of the female, while the Leghorn

male is male-feathered. The work of Morgan[1] (and later of Eliot, unpublished) shows that the castration of the male Sebright is followed by the formation of capon feathers (usually called male feathers); thus the testis of the Sebright, like the ovary of the hen, and unlike the testis of the Leghorn, inhibits the formation of male feathers. Morgan concluded from this result and from later histological studies, in conjunction with Miss Boring,[2] that the Sebright and the Leghorn testes have different endocrine secretions, and that the Sebright testis contains cells indentical with the so-called luteal cells of the ovary, the presumed source of the internal secretion of the latter, which are responsible for hen-feathering of the male. Thus the Sebright was regarded as hermaphroditic in respect to its endocrine apparatus. The relation of the so-called luteal cells of the testis to hen-feathering, however, has been denied by M. S. Pease,[3] Goodale, and Nonidez,[4] who arrived at the conclusion that there is no morphological difference between the testes of different breeds to account for a presumed difference in their endocrine functioning. This led Prof. F. R. Lillie to suggest that the problem be attacked experimentally. The work was done on

10 Autotransplantation in the Leghorn,
2 Autotransplantation in the Sebright,
50 Cross-transplantation of Sebright testis into the Leghorn capon,
38 Cross-transplantation of Leghorn testis into the Sebright capon.

Autoplastic grafts grow readily in both breeds, and, when sufficiently developed, substitute completely, both physiologically and psychologically, for the normal glands. This confirms the results of previous investigators. In such grafts normal spermatogenesis may proceed indefinitely.

The birds retained the original feathering, prominent head furnishings, and male sex instincts during the time the grafts were intact. The removal of the grafts was soon followed by the regression of the size of head apparel, loss of sex instincts and, in

[1] Morgan, T. H., *Carn. Inst. Wash. Pub.* No. 286, 1919. *Endocrin.*, 1920, iv, 381-386.

[2] Boring, A. M., and Morgan, T. H., *J. Gen. Physiol.*, i, 127-131.

[3] Pease, M. S., *Proc. Camb. Phil. Soc.*, 1922, xxxi, 22-36.

[4] Goodale, H. D., and Nonidez, J. F., *Am. Nat.*, 1924, lviii, 91-92.

the case of the Sebright, change of plumage from the henny to the capon type.

II. EXPERIMENTS ON CROSS-TRANSPLANTATION.

To find out if the Sebright and the Leghorn testes are different from each other in their endocrine functioning, and especially if their secretions have specifically different effects on plumage, the following experiments were performed:

1. Leghorn cockerels were castrated and Sebright testis implanted into them.

2. Sebright cockerels were castrated and Leghorn testis implanted into them.

1. *Transplantation of Sebright Testis into the Leghorn.*

Fifty Leghorn males were used, all of which were successfully castrated and received Sebright testis grafts. These grafts "take" with great difficulty in Leghorns and only two successful cases out of 50 capons were secured. In these cases (L. 95 and 155) the Sebright testis caused in the Leghorn the formation of typical prominent head furnishings and sex and fighting instincts of the male. However, the feathers remained typical Leghorn male whether replaced by normal moult or after removal by plucking which was practiced several times. There was no tendency to the production of hen-type feathers caused by the Sebright graft, though this was active enough to induce the typical male characters of head furnishings and behavior. In the one case, the graft remained for 3 months and 24 days (No. 95) and in the other for 4 months and 14 days (No. 155). On internal examination, the original testicular sites were found clean and free from any growth, and growing grafts were found at the original sites of implantation. As soon as the grafts were removed, the head furnishings immediately receded and the male sex and fighting instincts disappeared. In L. 155 the graft recovered contained pigmentary cells which furnished additional evidence that the tissue recovered is the same as the tissue implanted.

2. *Transplantation of Leghorn Testis into the Sebright.*

This form of cross-transplantation succeeds much more readily than the reciprocal. Eleven out of the 38 cases were successful. Nevertheless, the grafts do not take so well as the

autoplastic grafts. Of these 11 birds, 9 developed capons feathers and the head furnishings regressed to the capon condition after complete castration and before grafting. Under the influence of the Leghorn testis grafts, the head furnishings developed again and the male sex and fighting instincts reappeared. The feathers, also, after plucking or molting, instead of being replaced by capon or cock feathers, developed as henny feathers, the animals reverting to the hen-feathering normal for the Sebright cock. In all except two (S. 3 and 12) no regenerated testicular tissue was found. But in these exceptions, also, the feathers remained the henny type under the influence of the Leghorn testis grafts after the regenerated tissue was removed.

After the grafts were removed, after being retained for from 3 months to a year or more, the sex instincts disappeared, and the feathers again developed to the capon type, the so-called male feathers in the Sebright.

c. Discussion of Results.

These experiments show that it is much more difficult to transplant Sebright testis into the Leghorn than it is to transplant Leghorn testis into the Sebright; that while the Leghorn testis will readily take in a Sebright, the growth of a Sebright testis in a Leghorn is an exception rather than the rule. This difference in the reciprocals is very striking. It has been found that of the two breeds, the Sebright is more hardy and less subject to infections, although it is the one more difficult to operate on. The question of blood differences in the breeds should receive attention.

These experiments clearly indicate that there is no difference in the endocrine secretions of the Leghorn and Sebright testes contrary to what has been postulated by some workers. A Leghorn testis in a Leghorn body is accompanied by the appearance of the usual male type feathering, while the same testis in a Sebright body is accompanied by the appearance of the Sebright henny feathering. A Sebright testis in a Sebright body provokes the production of the henny type of feathers, while the same testis in a Leghorn body is accompanied by the production of the usual Leghorn feathers. It seems safe to conclude then that there is no qualitative difference in the secretion of the two testes as far as their effect on plumage is concerned, both producing the same effect when placed in the same body.

Hen feathering in the Sebright has been shown by Morgan[1] to be dominant to cock-feathering in crosses and to behave in a general Mendelian fashion. It is natural to conclude, therefore, at least provisionally, that the difference in the feather reactions to the testis hormones in the two breeds is based upon such differences in their genetic constitution, though other possibilities remain to be considered elsewhere. This implies that while the male type of head furnishings and the development of male sex instincts are determined in all their extent by the testicular hormone, the plumage of the male is conditioned in both breeds by another factor or set of factors, presumably genetic, in addition to the influence of the testis.

The results, in any case, remove the necessity of assuming two kinds of endocrine secretions by the Sebright testis, which is inherent in Morgan's theory. It may also be incidentally noted that there is no noticeable racial influence of the heteroplastic testis.

Minnesota Branch

University of Minnesota Medical School, May 5, 1926.

3174

The presence of vagus fibers in the splanchnic nerve of the cat.

A. T. RASMUSSEN and DONALD DUNCAN.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.*]

The recent announcement by Iwama[1] that a considerable number of medullated fibers from the vagus enter the sympathetic trunk near the inferior cervical ganglion, especially on the right side, and that some of these fibers run into the abdominal viscera via the splanchnic nerves, immediately brings into question considerable visceral neurology based upon experimental stimulation of the splanchnic and the vagus in the cat, at least. According to Iwama's observations it is impossible to stimulate the right splanchnic of the cat without also stimulating some vagus fibers.

It also occurred to us that there might be enough variability in number of vagus fibers that reach the gastric regions through the splanchnic nerves of different species of animals to be a factor in the results obtained by Carlson and others[2] following stimulation of the peripheral end of the splanchnic, such as only inhibition of the cardia in the rabbit and either contraction or inhibition in the cat depending on the state of tonus at the time of stimulation.

The preliminary work necessary to get this question upon a quantitative basis gave results at such variance with those expected that this note is deemed advisable although only four cats and two rabbits of our series bear directly on the subject.

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[1] Iwama, Y., *Folia Anatomica Japonica*, 1925, iii, 215.

[2] Carlson, A. J., *Am. J. Physiol.*, 1922, lxi, 14.

Section of the right vagus in the mid-cervical region of the rabbits produced no Wallerian degeneration in the thoracic sympathetic trunk and hence none in the splanchnic nerves.

In three cats (right vagus cut in two and left vagus in one) results were similarly negative. In a fourth cat, however, with right vagus cut above the anastomosis with the inferior cervical sympathetic ganglion, two degenerated medullated fibers were clearly identified in the right thoracic sympathetic trunk above the origin of the splanchnic nerve. One degenerated fiber was found in the right splanchnic and one in the right lumbar sympathetic trunk. Evidently one of the two vagus fibers coursed via the splanchnic and the other passed farther down the sympathetic trunk. Serial longitudinal sections and teased glycerol specimens agreed absolutely with each other. The glycerol specimens had never been in any fat solvent. Control material from corresponding nerves on the opposite side were run through the same reagents with the experimental specimens.

We do not believe these results are due to faulty technique, since one of us (A. T. R.) has used the Marchi method more or less continuously for over ten years and should realize its limitations. Hence we conclude that the number of medullated fibers from the vagus that course into the abdominal viscera via the splanchnic nerves in both rabbits and cats must be very small and therefore insignificant functionally.

3175

Inhibition of renal secretion following injury in the neighborhood of the colliculi.

F. H. SCOTT and M. M. LOUCKS.

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Desiring to obtain some data on the output of certain urinary constituents in dogs during short intervals of time, we thought we could get rid of the influence of anesthetics by decerebration. However, we were greatly surprised to find that on decerebration, the flow of urine ceased, although there had been a good flow

previously. There was no material alteration of blood pressure and respiration was kept up. Subsequent investigation showed this to be a constant phenomenon. Under normal conditions, after decerebration, there are a few drops of urine at the previous rate, and then a marked slowing in the rate amounting in most cases to a stoppage, and lasting as long as the experiment, in one case, seven hours after decerebration.

In our experiments, dogs have been used exclusively. They were given morphine and ether. Cannulae were inserted into the ureters close to bladder and the flow of urine recorded by allowing the drops of urine to fall on a lever which closed a circuit and thus recorded the flow by means of an electromagnet. Blood pressure was recorded from the left carotid, and the right carotid and the two vertebrals were usually ligated or clamped. The skull was trephined and the urine output recorded this way for a time. A few results may be given. Thus one kidney which produced 75 drops of urine in the thirty minute period previous to decerebration, produced 9 drops in the following thirty minutes. Another kidney which produced 138 drops in 30 minutes previous to decerebration, produced only 24 drops during 30 minutes following decerebration. Another kidney which formed 49 drops in 15 minutes previous to decerebration produced only 9 drops in the following 35 minute period.

Altogether, over thirty experiments have, so far, been done with similar results.

We believe the few drops of urine coming after decerebration at the previous rate were already in the ureter.

That this stoppage of renal secretion is a nervous phenomenon can readily be shown by sectioning the nerves going to one kidney before the operation. Decerebration then has little effect on the denervated kidney. In our experiments we found the flow of urine from the denervated kidney to be irregular and coming more or less in gushes. The results of one experiment may be given. Left kidney denervated.

Before decerebration:

Average drops per min. (20 min.) from left kidney.....2.2
Average drops per min. (20 min.) from right kidney.....2.3

After decerebration:

Average drops per min. (45 min.) from left kidney.....4.2
Average drops per min. (45 min.) from right kidney..... .5

It is noticed that the denervated kidney increased in activity while the kidney with uncut nerves was markedly slower than before the operation.

Section of one splanchnic nerve has a similar effect on the flow from the kidney of that side as section of the nerves to the kidney itself.

When the kidneys stop secreting as a result of decerebration, one can cause them to secrete by large doses of urea, or in most cases, by large doses of physiological saline intravenously, but one has to inject more saline to produce a diuresis than in normal animals. In a number of cases, injection of large amounts of saline produced only one or two drops of urine.

A study of the blood showed that the saline did leave the blood for the tissues but much more slowly than normal. After a dose of 20 cc. per kilo the blood of a normal animal returns to its original condition in from 30 to 35 minutes, but after decerebration little more than half has left at that time.

If one produces a diuresis before decerebration, either by intravenous injection of saline or by giving water by the mouth, then decerebration usually causes only a temporary slowing for a few minutes and then the secretion goes on at its former rate for a time and then ceases when the excess of fluid is removed.

The two things best known to affect the rate of kidney secretion are blood pressure and lack of oxygen. These two factors may be ruled out in these experiments. On decerebration there is, as a rule, immediately, either a slight rise or fall of pressure, lasting for a few minutes, and then a return to normal. There is usually increased pulse pressure after decerebration. Lack of oxygen cannot be the cause, because of the sudden onset of the effect. Experiments to test this point show a gradual slowing as asphyxia comes on. We believe the cause of this stoppage on decerebration lies in the altered permeability of the capillaries.

Various authors (Ashner, Camus and Roussy, Bailey and Bremer Curtis[1]) have described how injury to the brain stem in the region of the hypophysis (*Corpora mammilaria* Bourquin[2]) causes an increased output of urine. Destruction around the colliculi has the opposite effect. Our experiments show that one cannot get very far away from the colliculi and get this effect.

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1 Curtis, G. M., *Arch. Int. Med.*, 1924, xxxiv, 801. (Gives earlier literature).

2 Bourquin, H., *Am. J. Physiol.*, 1926, lxxvi, 181.

We hope soon to be able to give a more exact location of the center.

We wish to call attention to a very similar phenomenon connected with the *nervi erigens* described by Martin and Tainter.[3] After decerebration, stimulation of the *nervi erigens* failed to produce its usual effect. Section of the nerves did not abolish the inhibition. If one sectioned the nerve before decerebration there was no effect on the action of the nerve as a result of decerebration. We know of no studies dealing with the permeability of the capillaries during stimulation of the *nervi erigens,* but the probabilities are that erection would be accompanied by outpouring of fluid to the tissue spaces. Our results and those of Martin and Tainter seem to indicate that injury in the neighborhood of the colliculi throws the capillary cells into some kind of a fixed condition of lessened permeability. We believe this mechanism is involved in those conditions of anuria seen when calculi are passing down the ureters. We have not been able to remove this condition by stimulation of nerves, but our experiments along this line have been very few.

3176

Further observations on relation of glomerular function to phenolsulphonephthalein excretion in frog's kidney.

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Since Rowntree's Phenolsulphonephthalein Test[1] of renal function is used widely in clinical practice, it is important to determine whether Phenolsulphonephthalein and similar dyes are excreted through the glomeruli, through the tubules, or through both.

In previous communications, we,[2] and also Richards and

[3] Martin, E. G., and Tainter, M. L., *Am. J. Physiol.*, 1923, lxv, 139.

[1] Rowntree, L. G., and Geraghty, J. T., *J. Pharmacol. and Exp. Therap.*, 1910, i, 579; *Arch. Int. Med.*, 1912, ix, 284.

Wearn,[3] observing the frog's kidney by Richard's method, have demonstrated this dye in the fluid within the glomerular capsules of the frog, indicating clearly that in this animal the glomeruli excrete Phenolsulphonephthalein. We have also shown that the dye appeared in the lumen of the distal convoluted tubules and in the straight tubules about 5 to 10 minutes after it appeared in the glomerular fluid; and that the color within the tubules showed the dye present in a much more concentrated solution than in the glomerular capsule. We found further that if branches of the renal artery were ligated, no dye appeared in the lumina of the tubules in the areas in which there was no active circulation through the glomeruli. We regarded this as proof that Phenolsulphonephthalein is excreted by the glomeruli and is concentrated in the tubules by the reabsorption of water. Marshall and his collaborators[4] have objected to this interpretation because they have found that the cells of the convoluted tubules of the frog, seen on the dorsal surface of the kidney, are deeply stained with the dye; and they regard that as evidence that it is excreted by these cells. We have confirmed their objective findings but not their interpretations, since the presence of a dye within a tubule cell does not necessarily indicate that that cell is excreting it into the tubule.

In a previous communication we[2] have also shown that, contrary to the claims of Nussbaum[5] and of Woodland[6] and other observers, there is a definite anatomical anastomosis between the branches of the renal artery and those of the renal portal vein within the kidney, especially in the lower half, and that the pulsating stream from the artery and the continuous flow from the vein can be seen as separate currents within the same blood vessel. One would, therefore, expect that when the dye is injected

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[2] Bieter, R. N., and Hirschfelder, A. D., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 415; *Am. J. Physiol.*, 1924, lxviii, 326; *J. Pharmacol. and Exp. Therap.*, 1925, xxv, 165.

[3] Richards, A. N., *Am. J. Med. Sci.*, 1922, clxiii, 1. Wearn, J. T., and Richards, A. N., *J. Am. Med. Assn.*, 1923, lxxx, 1644; *Am. J. Physiol.*, 1924, lxxi, 209.

[4] Marshall, E. K., Jr., and Edwards, J. G., *Am. J. Physiol.*, 1924, lxx, 489; Marshall, E. K., Jr., and Crane, M., *Am. J. Physiol.*, 1924, lxx, 465; Marshall, E. K., Jr., and Vickers, J. L., *Bull. Johns Hopkins Hosp.*, 1923, xxxiv 1.

[5] Nussbaum, *Arch. f. d. ges. Physiol.*, 1878, xvi, 139; *ibid.*, 1878, xvii, 580.

[6] Woodland, J., *J. and Proc. Asiatic Soc.*, 1922, N. S. xviii, 85.

into the renal portal vein some of it would enter the glomeruli and be excreted by them.

Further observations as to glomerular activity obtained by excretion of phenolsulphonephthalein and the resulting injection of these glomeruli by American India Ink gives the results described below.

The technique for these procedures is as follows: Large frogs, weighing from 200 to 650 grams, of the species *Rana Catasbiana,* are anesthetized with urethane. The frogs are then tied down with the ventral surface upwards and the abdominal cavity opened by the method described elsewhere.[2] By means of a series of cannulae the urine from the upper and lower halves of the kidney can be collected separately. With another set of cannulae arterial blood can be transported from an aortic branch to a common iliac so that this blood when collected by the veins can be run into the kidney through the renal portal system. Oxygenated Ringer's solution is now run into the renal arteries so that, as we have found, it will strike most of the glomeruli of the upper half of the kidney, and there be filtered to produce a fluid in the urinary tubules. Phenolsulphonephthalein solution and American India Ink can now be injected into the system of cannulae carrying blood around the kidney so that it can return through the kidney by means of the renal portal system, or it can be injected into a branch of the renal portal vein.

With this technique the following results have been obtained: In a series of 10 frogs when Phenolsulphonephthalein has been injected into the blood being carried around the kidney to return as renal portal blood, the urine in the lower cannula shows Phenolsulphonephthalein within five or ten minutes, and the urine in the upper cannula shows Phenolsulphonephthalein in from ten to thirty minutes or in some cases not at all. The lower cannulae always show the Phenolsulphonephthalein in a deep concentration and in a fairly large amount, whereas the upper shows only in traces.

In another series of five frogs treated as above except that the Phenolsulphonephthalein and India Ink solution was injected continuously through the experiment into the anterior abdominal or pelvic vein on the left side, the lower cannula showed Phenolsulphonephthalein in an amount from two to four or five times as concentrated as the original solution injected, whereas the upper cannula showed the dye only in traces and never as concen-

trated as the original solution. The amounts of urine from the upper and lower halves ran fairly uniformly—*e. g.*, from one frog being 0.23 cc. for the upper portion and 0.26 cc. for the lower portion.

These kidneys removed after the experimental procedure, fixed, sectioned, and stained with eosin, showed (1) grossly, a kidney fairly uniformly injected with ink, and (2) microscopically, a fair degree of uniformity of tubular capillary injection and glomerular injections of upper and lower halves as follows: in the upper portion of the kidney from 4 to 32 per cent of the glomeruli were injected with an average of 14.3 per cent, whereas in the lower portion, from 17.5 to 97.5 per cent were injected, with an average of 43.1 per cent.

In the light of these findings, the burden of proof in the question as to whether the dye is excreted by way of the glomeruli or by way of the tubules, lies with those who claim that it is excreted by way of the tubules.

SUMMARY.

When a dye solution is run into the frog's kidney in such a way that, in the lower portion of the kidney, it runs into tubular capillaries and into a large number of glomerular capillaries, while in the upper portion of the kidney it runs into tubular capillaries, and into only a few glomerular capillaries, the amount of dye excreted follows the number of active glomeruli receiving dye solution, although both halves of the kidney are producing urine.

3177

On correlation between age of parents and length and weight of the newborn infant.

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[*From the Department of Botany, University of Minnesota, Minneapolis, Minnesota.*]

It seems reasonable to assume that a number of factors influence the size of the human individual at various stages of

*These investigations were begun while the writer was a member of the staff of the Station for Experimental Evolution and their completion has been facilitated by a research grant from the Carnegie Institution of Washington.

development. Pearson and his co-workers have shown the great importance of hereditary factors. They have found that in general such environmental factors as they have been able to consider play a much smaller rôle in determining the characteristics of the individual. Nevertheless it seems important to investigate all such factors in as great detail as possible, with a view to disentangling those factors which are innate in the zygote from which the individual develops from those which are extrinsic to the individual, and to measuring quantitatively the relative importance of each. It seems especially desirable to consider certain morphogenetic factors.

The purpose of this paper, which is one of a series dealing with the physical characteristics of the newborn infant of various races, is to consider the possible relationships between the age of the parents and the length and weight of the newborn child.

The data represent measurements on infants of the white race taken at the Sloane Hospital for Women, New York City, and recorded in the archives of the Obstetric Divisions of that institution. For opportunity of using these records we are indebted to the late Dr. W. E. Studdiford, who was Superintendent at the time this phase of the investigation was undertaken.†

Since it is possible that the average age of parents and the average dimensions of the newborn infant differ from nationality to nationality, we have considered separately the characteristics of infants born of Austrian, English, German, Irish, Italian and Russian parents. Both parents were born in the countries designated. By the examination of about 44,000 records assembled over a period of about 30 years (September, 1890, to September, 1921), it was possible to secure the following numbers of measurements of length and weight.

	Male	Female
Austrian	117	136
English	170	158
German	443	402
Irish	1138	1071
Italian	126	123
Russian	324	301

† This work was begun with Dr. C. C. Little, who was forced to give up personal cooperation at an early stage due to the pressure of administrative duties at the University of Maine and subsequently at the University of Michigan.

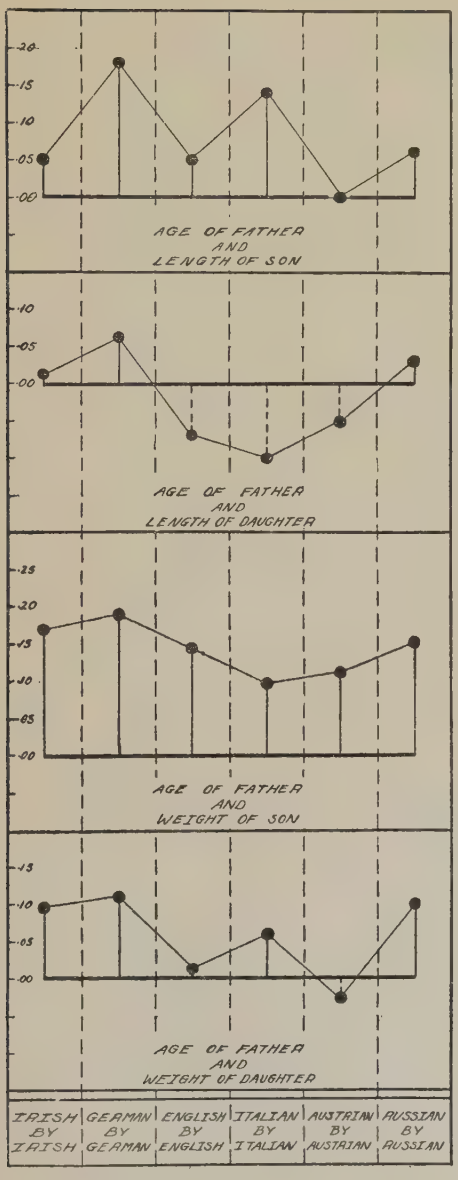

DIAGRAM 1.

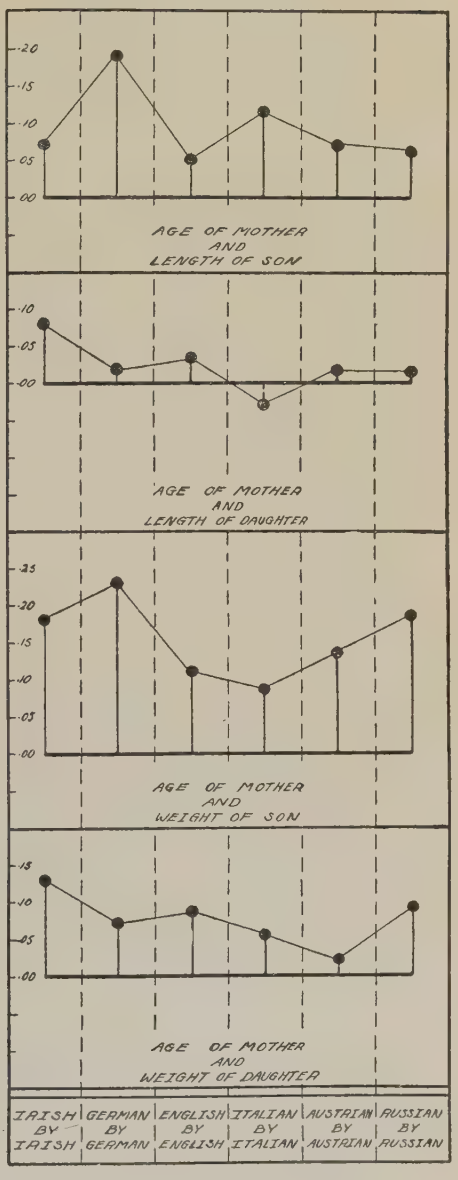

DIAGRAM 2.

The interrelationship between the age of the parents and the length and weight of the infant has been expressed in terms of the correlation coefficient. For present purposes a graphic method of presenting the constants is sufficient. They will be given in full and considered in relation to their probable errors in a more extended publication. In Diagram 1, for the relationship between age of the father and the length and weight of the son and daughter, and in Diagram 2 for the relationship between the age of the mother and the length and weight of the son and daughter, the heavy transverse bars in the four panels denote the position of zero correlation as shown on the scale of ordinates.

Considering first the relationship between the age of the father and the characteristics of the infant, we may note that all of the correlations between age of father and length of son are positive and that 5 are larger than 0.05. In the case of the relationship between the age of the father and the length of the daughter 3 of the coefficients are positive while 3 are negative in sign. All are of a very low order of magnitude. We may not, therefore, conclude that there is any very intimate relationship between the age of the father and the length of the child.

All of the coefficients indicate that the relationship between the age of the father and the weight of the son is positive and of the order +0.10 or higher. The coefficients measuring the relationship between age of the father and the weight of the daughter are positive in 5 of the 6 series.

While these coefficients are generally low, they are prevailingly positive in sign. Taking all of the relationships together, 20 of the 24 coefficients are positive as compared with 4 which are negative. On the average the positive coefficients are larger than those which are negative. This furnishes evidence for the existence of a *slight* positive correlation between the age of the father at the time of the birth of the child and its physical dimensions.

The average values of the coefficients for the six series are:

Age of father and length of son, $\overline{r} = +.0828$

Age of father and length of daughter, $\overline{r} = -.0187$

Age of father and weight of son, $\overline{r} = +.1464$

Age of father and weight of daughter, $\overline{r} = +.0591$

The negative value is sensibly zero. The others are low order positive values.

Turning to the relationships for the mother, we note that all of the correlations between the age of the mother and the length and weight of son are positive. Most of them are of the order 0.10 or higher. The coefficients measuring the relationship between the age of the mother and the length and weight of the daughter are positive in 11 of the 12 series. Taking the data as a whole, it appears that 23 of the coefficients are positive as against 1 which is negative.

The average values of the correlation coefficients measuring the relationship between the age of the mother and the characteristics (length and weight) of her child are:

Age of mother and length of son,	$\overline{r} = +.0948$
Age of mother and length of daughter,	$\overline{r} = +.0245$
Age of mother and weight of son,	$\overline{r} = +.1563$
Age of mother and weight of daughter,	$\overline{r} = +.0776$

While these values are very low, the consistency of the results can leave little ground for doubting the conclusion that there is a definite relationship between the age of the mother and the length and weight of her newborn infant. The averages are slightly higher than those for age of father.

The indications of a positive correlation between the age of the father and the characteristics of the infant should not lead to the conclusion that there is any direct morphogenetic relationship between the age of the father as such and the length and weight of the child. The ages of father and mother are closely correlated. Thus if there be any definite relationship between the age of either of the parents and the characteristics of the child there should be some correlation between the age of the other parent and the dimensions of the child due solely to the age correlation of the parents themselves.

Neither can we conclude that there is a direct causal relationship between the age of the mother and the characteristics of the child. In a subsequent paper it will be shown that there are positive correlations between birth order and pregnancy order and the measurements on the infant. Both birth order and pregnancy order are themselves intimately correlated variables and both are closely though less highly correlated with the age of the mother.

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Relationship between pregnancy order and birth order and length and weight of newborn infants.

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In a preceding paper[1] evidences for the existence of a low positive correlation between the age of the parents and the length and weight of the newborn infant of various nationalities have been adduced. The present paper is devoted to a consideration of the relationship between pregnancy order and birth order on the one hand, and the length and weight of the infant on the other. The source of the data is the same as that of those employed in the preceding investigation.

By pregnancy order I understand merely the serial order of the pregnancy (Gravida). By birth order I understand merely the order of birth of the child (Para). These differ by the number of miscarriages which may have occurred previous to the birth under consideration.

In Diagram 1 and 2 the heavy bars in each of the four panels denote zero correlation, as shown on the scale of ordinates.

Examination of the correlations for pregnancy order and the lengths of boy and girl infants shows that in 9 of the 12 available series the coefficients are positive, whereas in 3 of the series they are negative in sign. For the relationship between pregnancy order and weight of child, all of the coefficients are positive in sign. Thus for both weight and length, 21 of the 24 coefficients indicate a positive correlation between pregnancy order and the weight or length of the child.

The average values of the coefficients measuring the relationship between pregnancy order and the characteristics of the child are:

Pregnancy order and length of son,	$\overline{r} = +.0914$
Pregnancy order and length of daughter,	$\overline{r} = -.0117$
Pregnancy order and weight of son,	$\overline{r} = +.2102$
Pregnancy order and weight of daughter,	$\overline{r} = +.1393$

* These investigations were begun while the writer was a member of the staff of the Station for Experimental Evolution, and their completion has been facilitated by a research grant from the Carnegie Institution of Washington.

[1] Harris, J. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 801.

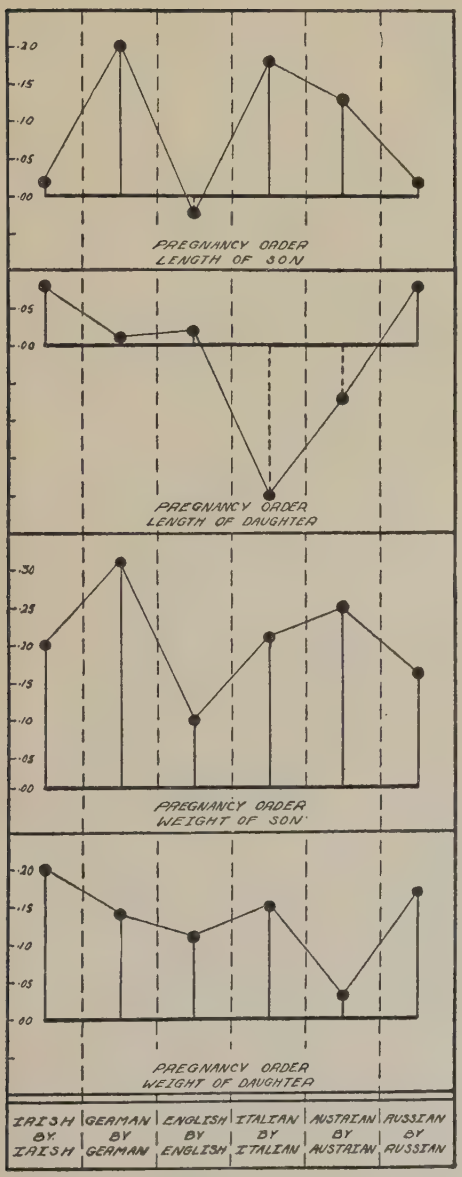

DIAGRAM 1.

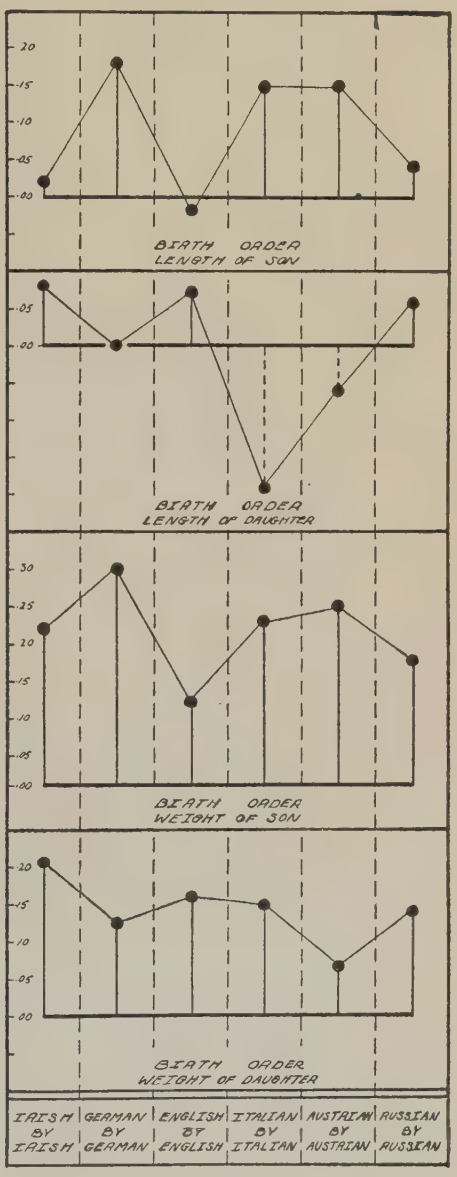

DIAGRAM 2.

The average of the coefficients for pregnancy order and length of girl infants is sensibly zero. The other averages are small, but sufficiently large to be considered probably significant.

Turning to the correlations showing the relationship between birth order and the length of the infant, we note a closely parallel condition. Of the 12 coefficients, 9 are positive as compared with 3 which are negative. In the case of the relationship between birth order and weight, the coefficients are without exception positive in sign and a number are of the order + .20 or larger.

In this series of coefficients there are certain outstanding exceptions to the prevailingly positive nature of the coefficients. These are seen especially in the case of Italian and Austrian girls. No explanation can be suggested for these cases.

The average values of the correlation coefficients are:

Birth order and length of son,	$\overline{r} = +.0900$
Birth order and length of daughter,	$\overline{r} = -.0054$
Birth order and weight of son,	$\overline{r} = +.2179$
Birth order and weight of daughter,	$\overline{r} = +.1434$

Again the average value for the girl infants is sensibly zero.

Considering these results in connection with those for the relationship between age and the characteristics of the infants as shown in the preceding paper, we may conclude that the evidence for some relationships between age of parents and pregnancy order and birth order, on the one hand, and the length and weight of the newborn infant, on the other, is unmistakable. We cannot, however, on the basis of the present data determine which of the four variables considered is the one which should be regarded as of primary importance in determining these interrelationships. This problem is being investigated on the basis of other series of data and in cooperation with embryologists and obstetricians.

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The prenatal growth and natal involution of the human suprarenal gland.

RICHARD E. SCAMMON.

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Two organs of the human body undergo a remarkable reduction in size following birth, these are the uterus and the suprarenal glands. The neonatal loss in mass of the suprarenals seems to have been first noted by Scheel.[1] It is due in great part, if not entirely, to a degeneration of the inner and middle layers of the cortex. This process was first described by Starkel and Wegrzynowski[2] and, shortly after, independently by Thomas,[3] Kern,[4] and Armour and Elliott.[5]

It has been pointed out that the neonatal loss in size of the uterus is preceded by a period of marked growth of this viscus in the latter part of fetal life, and that this neonatal involution reduces the organ to essentially the dimensions which would have obtained had the early fetal growth rate remained unchanged. Since the suprarenals undergo a neonatal involution which is concomitant with that of the uterus, it seems desirable to determine whether they also show a period of increased growth in the latter part of prenatal life.

The present study is based on observations on the weights of 1087 pairs of suprarenal glands; 425 of fetuses, 338 of newborn infants, stillborn or dying within 2 days after birth, and 324 of children over 2 days and under 1 year of age.

The course of growth of these structures is shown in the accompanying figure in which the suprarenal weight is plotted against the total body weight (as indicated on the base line scale) and the computed prenatal age and observed postnatal age, as indicated on the upper boundary line of the graph. The ob-

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1 Scheel, O., *Arch. f. path. Anat.*, 1908, cxcii, 494.

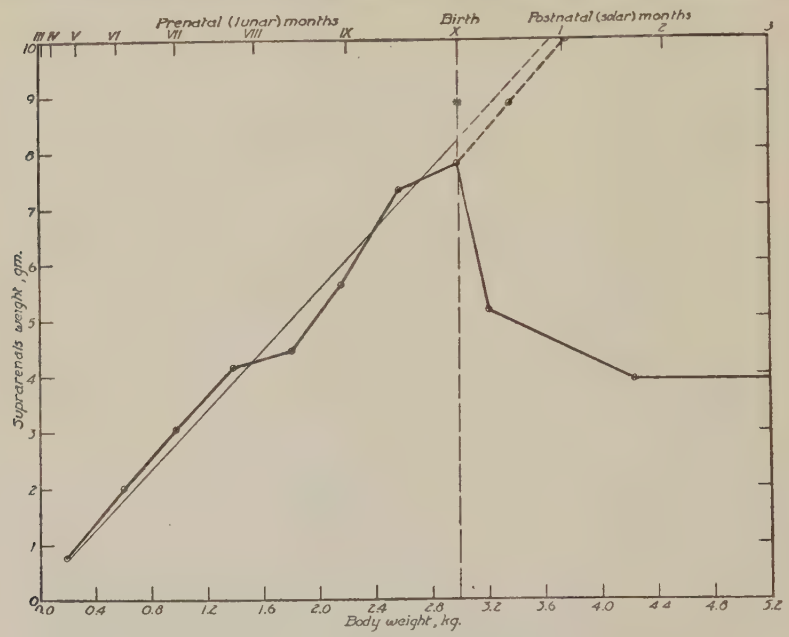
2 Starkel, S., and Wegrzynowski, L., *Arch. f. Anat. u. Entwickelungsges*, 1911, 191.

3 Thomas, E., *Beitr. z. path. Anat. u. allg. Pathol.*, 1911, l, 283.

4 Kern, H., *Deutsch. med. Wochenschr.*, 1911, xxxvii, 971, 1180, 1318.

5 Armour, R. G., and Elliott, T. R., *J. Path. and Bact.*, 1911, xv, 481.

servations on fetal material indicate that the growth of the suprarenals before birth is proportional to the growth in body-weight for the relation between these weights is approximately rectilinear



A graph illustrating the ponderal growth of the human suprarenals in prenatal life and infancy. The weight of the suprarenals (in grams) is plotted against the total body-weight (in kilograms) and the computed age (in lunar months for the fetal period and in solar months for postnatal life). The body-length is indicated on the base line of the graph and the computed age on the upper line. The age in the fetal period is computed according to the "exact" empirical formula of Scammon and Calkins.[6] The relation between body-weight and age in postnatal life is computed from the formula of van Leer,[7] with the initial value changed from 3250 grams to 3000 grams to make the results comparable with those obtained in this series. The circles, in the fetal period, represent the mean values for 400 gram intervals of body-weight, that at 8 postnatal days represents the mean value for 48 infants between 2 and 14 days of age, and that in the second month represents the mean value for 78 infants between 2 weeks and 3 months in age. The star represents the mean value for all material reported in the literature and in our own records as "newborn", regardless of body-weight or body-length. The heavy solid line is the point to point curve of observed values. The heavy broken line is the point to point curve of observed values for "postmature" infants (over 3200 grams dead-weight at birth). The light solid line is the computed curve for growth in the weight of the suprarenals with respect to body-weight in the fetal period. The broken light line represents the continuation of this curve for "postmature" infants.

[6] Scammon, R E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 157.

[7] van Leer, S. A., *Arch. f. Kinderheilk.*, 1902, xxxiv, 293.

throughout the fetal period. An empirical formula has been fitted to these data by the method of least squares on the basis of the mean weight of the suprarenal for each 400 gram interval of total body-weight. The values were weighted by the square root of the number of cases in each interval. The expression obtained is:

$$S.\ W. = 2.704\ B.\ W. + 0.13$$

where "S. W." is the weight of both suprarenal glands in grams and "B. W." is the total dead body-weight in kilograms. The calculated values obtained by this formula for the 400 gram intervals of body-weight show a mean deviation, taken without regard to sign, of 0.32 grams, from the corresponding observed values.

The course of the postnatal involution of the suprarenals is indicated by a point to point curve based on averages for the material arranged in two groups according to age; the first including all cases between 2 and 15 days, and the second, all cases between 15 days and 3 months. The decrease in weight amounts to approximately one-third of the natal weight in the first fortnight and to approximately one-half of the natal weight in the first trimester after birth.

The growth of the suprarenals in so-called "postmature" fetuses (as indicated by specimens above the calculated dead weight of the body at birth—see explanation of figure) continues at approximately the same rate as in the earlier part of the fetal period.

These data indicate that the suprarenals, unlike the uterus, show no phase of augmented rate of growth, with respect to the growth of the body as a whole, in the latter part of fetal life, preceding their neonatal involution.

Miscellaneous*

3180

Studies on experimental cretinism. II. Nutritional disturbances of bones.

MARGARETE M. KUNDE* and L. WILLIAMS.

[*From the Department of Physiology, University of Chicago, Chicago, Ill.*]

Rabbits and rats on an adequate diet and thyroidectomized 2 to 3 weeks after birth show disturbances in the development of bones during the growing period, which appear identical with the pathological processes ascribed to rickets.

3181

Studies on experimental cretinism. III. Nutritional disturbances, pellagra and xeropthalmia.

MARGARETE M. KUNDE.*

[*From the Department of Physiology, University of Chicago, Chicago, Ill.*]

Eight to twenty months after complete thyroidectomy in the rabbit, well defined skin lesions appear on the dorsum of the feet, which have the characteristics of the lesion ascribed to pellagra. Pellagra-like lesions may also be apparent around the neck and in a circumscribed area about the mouth and nose. Xeropthalmia may also occur. These deficiencies appear in the hypothyroid rabbits on diets that are adequate for normal rabbits.

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* Received too late to be placed in their proper order.

* Douglas Smith Fellow in Physiology.

3182

Studies on experimental cretinism. IV. The influence of thyroidectomy on the central nervous system.

MARGARETE META KUNDE.*

[*From the Department of Physiology, University of Chicago, Chicago, Ill.*]

Rabbits thyroidectomized 2 to 3 weeks after birth have been allowed to develop in a hypothyroid condition for 10 to 16 weeks. At that time marked symptoms of cretinism are apparent. These rabbits have then received daily doses of dessicated thyroid by mouth or intravenous injections of Kendall's thyroxin, until the end of the growth period. If the thyroid treatment is then discontinued and the animal allowed to live for a long period of time, usually eight months or more, the posterior extremities become spastic. Reflexes are exaggerated and a slowly progressive paralysis occurs, which results in complete inability to use the hind legs. Histological study of the spinal cord shows degenerative changes. The blood picture is that of primary anemia.

3183

Quinin in paroxysmal auricular tachycardia.

JOHN H. WYCKOFF and HAROLD L. OTTO. (Introduced by Holmes C. Jackson).

[*From the Department of Electrocardiography, New York University and Bellevue Medical College, New York City.*]

Wenckebach,[1] in an article upon cinchona derivatives in the treatment of heart disorders, stated that "an intravenous injection of quinin may stop an attack of paroxysmal tachycardia in many cases." This therapeutic suggestion was tested 10 times among 5 patients suffering from organic heart disease and paroxysmal auricular tachycardia, during a paroxysm. In all cases the effect of posture and the various forms of vagal pressure were

* Douglas Smith Fellow in Physiology.

1 Wenckebach, C., *J. Am. Med. Assn.*, 1923, lxli, 472.

first essayed. This was without avail in all. Continuous electrocardiography upon a lead selected from the diagnostic electrocardiogram was begun before the injection commenced and continued until 2 minutes after its completion. The injection occupied about 5 minutes. The dihydrochloride salt of quinin in 0.3 to 0.6 gram doses diluted to 10 cc. was used.

In six injections among four of these patients there was no alteration produced in the tachycardial rhythm governing the heart beat. In the fifth patient, the attacks came to an end with a single auricular premature contraction preceding the onset of the normal sinus rhythm, approximately one and one-half minutes after the injection was completed. This was repeated in three more paroxysms. This patient was having paroxysms lasting about one hour and a half four to six times a day. The injection of the drug did not lessen the frequency of the attacks. In one instance, not included among these ten, a paroxysm came to an end just as the quinin was about to be administered. This points to the caution with which inferences regarding the positive effects of therapy in this condition must be surveyed. In all of these patients no more paroxysms appeared after the oral administration of quinidin.

3184

Clinical action of adrenalin upon premature contractions.

HAROLD L. OTTO. (Introduced by Holmes C. Jackson).

[*From the Department of Electrocardiography, New York University and Bellevue Medical College, New York City.*]

Among 12 patients with varying types of heart disease presenting persistent premature contractions, including all the important etiologic, anatomic, and function combinations, epinephrin in a dose of 1 cc. in a solution of 1-1000 was injected hypodermically 18 times. Electrocardiograms of one minute duration on a previously selected lead were taken every five minutes; five before and five or more after the injection.

A considerable increase in the average number of premature contractions occurring per minute resulted in all cases begin-

ning five minutes after the injection and enduring thirty minutes to one hour. Rise in blood pressure and slight increase in the heart rate in most instances accompanied this. Those with auricular premature contractions frequently developed ventricular premature contractions and vice versa, where they were not in evidence before the injection. Those with left ventricular premature contractions often developed right ventricular premature contractions and vice versa. Two or three ventricular premature contractions occurring together were not infrequent. In each patient with auriclar premature contractions, groups of four and five premature contractions in succession appeared. In one individual, free of premature contractions by means of quinidine, many appeared following it. In another, freed of premature contractions by means of digitalis, none appeared following injections of epinephrin. Apparent rhythmicity in the recurrence of the premature contractions often occur following injection. No variation from the basic normal sinus rhythm predominating ever occurred.

3185

Calcium as a diuretic.

HAROLD L. OTTO. (Introduced by H. C. Jackson).

[*From the Department of Electrocardiography, New York University and Bellevue Medical College, New York City.*]

Singer[1] reported upon the favorable therapeutic effect of calcium, administered intravenously, in conjunction with digitalis in the conjestive type of heart failure.

Segall and White[2] concluded that calcium chloride may be employed as a diuretic in this type of heart failure.

Eight patients with congestive heart failure in whom rest in bed and digitalis did not completely remove the edema present, were treated with 15 grams of calcium chloride daily, administered orally, in divided doses, over periods varying from one week to 10 days. No significant changes followed in heart rate,

[1] Singer, G., *Wien. klin. Wchschr.*, 1921, xxxiv, 247.

[2] Segall, H. N., and White, P. D., *J. Am. Med. Soc.*, 1925, clxx, 647.

blood pressure, electrocardiography, urinary output or body weight. In five of these, marked diuresis and significant loss of body weight with disappearance of the edema present followed the administration of other diuretics. In one of these, another diuretic was tried both before and after the trial of the calcium with excellent response at both times, though none occurred during the period of exhibition of the calcium. (This individual only became edema-free when treated with the combination of rest in bed, digitalis and diuretics. When the diuretics were withdrawn, the edema of the dependent portions of his body gradually returned.)

In two more patients with congestive heart failure of similar functional condition, given daily injection of calcium chloride in 0.5 gram doses, results similar to those obtained by oral administration of the calcium occurred.

3186

The protective action of quinidin against the onset of paroxysmal auricular fibrillation and tachycardia.

HAROLD L. OTTO. (Introduced by H. C. Jackson).

[*From the Department of Electrocardiography, New York University and Bellevue Medical College, New York City.*]

(1) A female, age 23, with mitral stenosis and insufficiency of rheumatic etiology, predisposed to paroxysms of auricular tachycardia, never failed, when not previously subjected to the influences of other drugs, to have a paroxysm of auricular tachycardia following 2 injections of 1 cc. of epinephrin, 15 minutes apart.[1] When quinidin sulphate, 0.2 grams, twice daily, was administered previously, a paroxysm failed to appear upon exhibition of epinephrin in the same manner. This was performed twice. The first time after 6 weeks of this quinidin therapy, and 7 days later during which time no quinidin was administered, a paroxysm of tachycardia followed the exhibition of epinephrin as described. Fourteen weeks of quinidin therapy followed this and at the end of this time no paroxysm appeared after the

[1] Otto, H. L., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 550.

epinephrin, yet five days later during which time no quinidin was administered, a paroxysm of tachycardia followed administration of the epinephrin.

(2) A male, age 65, with arteriosclerotic heart disease, subject to attacks of paroxysmal auricular fibrillation during a period of normal sinus rhythm, was similarly treated with epinephrin. Auricular fibrillation appeared with very little change of rate about 15 minutes after the 2nd injection of epinephrin. Quinidin was utilized to re-establish the normal sinus rhythm, which reappeared 12 hours after administering 0.2 grams every two hours. The dosage was then reduced to 0.2 grams four times daily. After four days administration of quinidin in this manner, during which time the normal sinus rhythm prevailed, epinephrin exhibited in the same manner was not followed by alteration of the rhythm controlling the heart beat. It appears as if quinidine offers a protection to the heart against the inception of paroxysmal auricular tachycardia and fibrillation induced by means of epinephrin.

3187

Nitrogen balance on a low protein diet in a case of diabetes mellitus.

W. S. McCLELLAN and R. R. HANNON.

[*From the Russell Sage Institute of Pathology and the Second Medical Division of Bellevue Hospital, New York City.*]

As low protein diets have been recommended in treatment of diabetes by Joslin,[1] Petren, as quoted by Joslin,[2] Newburgh and Marsh,[3] and others, a study of long continued low protein diet was made in this case. The patient was a man, age 32, with a history of moderately severe uncomplicated diabetes for the past 2½ years.

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[1] Joslin, E. P., "Treatment of Diabetes Mellitus," 3d ed., Lea and Febriger, 1923, p. 444-6.

[2] *Ibid.*, p. 525-32.

[3] Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1920, xxvi, 647; 1922, xxix, 97; 1923, xxxi, 455.

Observations extended over a period of 6 months. The diets were carefully weighed and the nitrogen in the food calculated from the Atwater tables. No analyses of food were made. Total nitrogen in the urine was determined daily by the Kjeldahl method. Nitrogen partition products were not determined. Nitrogen in stools was determined for two ten day periods by the same method. Daily weights were recorded.

The patient received a diet of 2000 calories with 50 grams of protein for 15 days before reduction of protein was started. The protein was gradually lowered by drops of 10 grams each until a 20 gram level was reached. Three or 4 days were allowed at each level for the patient to adjust himself to his new diet. The patient remained at this level for 106 days. The calories remained the same during the entire period. Carbohydrate and fat were changed from time to time so that the fatty acid-glucose ratio varied between 0.9 and 3.0. In this change the carbohydrate varied from 132 grams to 30 grams in successive steps, the calories being replaced by fat. The nitrogen excretion was not noticeably affected. This suggested that fat served to spare protein as efficiently as carbohydrate. Insulin was required during the entire period. There was no glycosuria. Moderate acetonuria appeared on fatty acid-glucose ratios above 1.5. The patient was in bed during the entire period.

Periods (days)	Av. weight Kg.	Av. nitrogen in food gm.	Av. nitrogen in urine gm.	Av. nitrogen in stools gm.	Av. total nitrogen excreted gm.	Nitrogen balance gm.
I (21)	44.29	3.20	3.24	0.75	3.99	—0.79
II (24)	44.18	3.18	2.84	0.75(?)	3.59(?)	—0.41
III (31)	43.93	3.21	2.91	0.75(?)	3.66(?)	—0.45
IV (30)	43.85	3.22	2.49	0.45	2.94	+0.26
Total (106)	44.06	3.20	2.87	0.60	3.47	—0.27

The general condition of the patient remained good. He took the diets without any complaint. There was no significant change in weight. There was a positive nitrogen balance until the 20

gram level was reached. The variations in nitrogen balance for the period of observation are presented in the following table. A low protein diet was followed for a period of 106 days without any evident ill-effect upon the patient.

3188

Early cirrhosis of the liver produced in dogs by carbon tetrachloride.*

PAUL D. LAMSON and RAYMOND WING.

[*From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.*]

In studying the toxicity of carbon tetrachloride it was found that this drug in pure form causes very severe central necrosis of the liver which heals with scar formation.[1, 2] The toxicity of a large dose of carbon tetrachloride (4 cc. per kilo) is greatly increased by the addition of alcohol.[3] Experiments were carried out to determine the effect of repeated doses of carbon tetrachloride alone and carbon tetrachloride given with alcohol in order to study the toxicity of such repeated doses and the effect on the liver. Ten dogs were used; some were given the therapeutic dose of carbon tetrachloride (3 cc.), others 4 cc. per kilo, and others the same doses of carbon tetrachloride but with the addition of approximately 25 cc. of 50 per cent alcohol, and finally a control series was given the same dose of alcohol alone. The dogs were given these doses of the drug over a period of approximately sixteen weeks. No signs of intoxication in any of the dogs were seen. They maintained or gained weight and were killed for autopsy in apparently perfect condition. (One or

* The funds for carrying out this work were given by the International Health Board.

1 Pessoa, S. B., and Meyer, J. R., *Boletin da Sociadade de Medecina e Cirurgia de São Paulo*, Brazil, 1922.

2 Gardner, George H., Grove, R. C., Gustafson, R. K., Maire, E. D., Thompson, M. J., Wells, H. S., and Lamson, Paul D., *Bull. Johns Hopkins Hospital* 1925, xxxvi, No. 2.

3 Lamson, Paul D., Gardner, George H., Gustafson, R. K., Maire, E. D., McLean, A. J., and Wells, H. S., *J. Pharm. and Exp. Therap.*, 1925, xxii, 215.

two dogs died from unknown causes within the first day or two.) The livers of the dogs receiving alcohol showed nothing macroscopically or microscopically. All the other dogs showed definite signs of an early cirrhosis of the liver. Macroscopically the liver showed a uniformly granular scarred surface in which there were numerous larger nodules and hyperplastic hepatic cells. Microscopically there was extensive scarring of the liver and distortion of lobulation by fibrous tissue. There was also abundant cellular infiltration and some regeneration of bile ducts. There was no apparent difference in the livers of the dogs receiving carbon tetrachloride alone or both carbon tetrachloride and alcohol.

It is hoped that the continuance of such administration of carbon tetrachloride will produce a true Laennec cirrhosis with circulatory obstruction.

These experiments will be reported more fully in *The Journal of Pharmacology and Experimental Therapeutics.*

Iowa Branch

State University of Iowa, April 28, 1926.

3189

Effect of irradiated winter milk and cod liver oil on growth of young of milk-fed rats.

AMY L. DANIELS, SARAH IDELL PYLE and LELA BROOKS.

[*From the Department of Nutrition, Iowa Child Welfare Research Station, State University of Iowa, Iowa City, Iowa.*]

During an investigation pertaining to the mineral deficiencies of milk, it was observed that the young, born of milk fed rats in February and March, 1926, were smaller, and their rate of growth was far less rapid, than that of any which we had heretofore noted in a long series of milk fed animals. These young were furthermore much smaller than young born of these same mothers during October and September, 1925. The appearance of the young in all groups (6) were so strikingly similar, even though the milk modifications were somewhat unlike, that it seemed as if the difficulty must be inherent in the milk, and not in the added inorganic substances. This was further emphasized by the fact that in two cases in which the mother rats were being used as controls, the animals were receiving only milk with the customary iron and iodine additions.

To determine what constituent of this particular "winter" milk was lacking, we reduced the number of young in each litter to four, and added to the milk rations of the mothers the following substances respectively: $Ca_3(PO_4)_2$, Vitamin B preparation, Yeast, and cod liver oil. In no case did we get satisfactory growth. Slight gains were observed with three of the cod liver oil groups. One group receiving the cod liver oil addition died, as did also the group receiving the $Ca_3(PO_4)_2$.

Besides the small and spindling appearance of these young, it was observed that the skin had a peculiar pink, yellow color, and

the fur was very thin and peculiarly wiry. In spots it was quite gone, giving the animals a moth-eaten appearance. A somewhat similar condition in the fur of animals receiving a purified ration, containing cotton seed oil for its chief fat component, seemed to have been corrected by irradiating the food mixture with ultra violet light from a quartz mercury vapor lamp. Therefore, it occurred to us that a similar procedure with our milk was worth trying. We were a bit skeptical as to the effect of such procedures, since the cod liver oil addition (1 cc. per day*) had been of little benefit, and since in animals receiving all milk, it did not seem possible that the anti-rachitic properties of the diet would be so low as to produce the stunted appearance. Furthermore, neither mother nor offspring had any of the gross symptoms of rickets. However, the very rapid improvement of the young in all of the groups receiving the irradiated milk[1] left no room for doubt regarding the effectiveness of the treatment. Growth was at once resumed. The appetite of the animals increased. The fur became thick, soft, and silky, and in a very short time the animals gave every appearance of being normal.

A study of the growth curves of the young of milk fed rats during other seasons, for example, July and August, and October and November, gave no such picture, nor did the growth curves of young reared on our milk rations in March, 1922, show such very great variations. It seemed, therefore, that the low growth promoting qualities of the February, March and April (1926) milk tested may be explained in part by the unusually long cloudy winter.[2] Numerous investigators have reported an increase in the anti-rachitic potency of milk by direct radiation,[3] and Hart,

* An approximate measure only. During the first days of the experiment the cod liver oil was added to the milk, all of which was not taken. The oil was then incorporated in a starch paste and fed as such.

[1] The milk was irradiated for one-half hour at a distance of two feet with a quartz mercury vapor lamp.

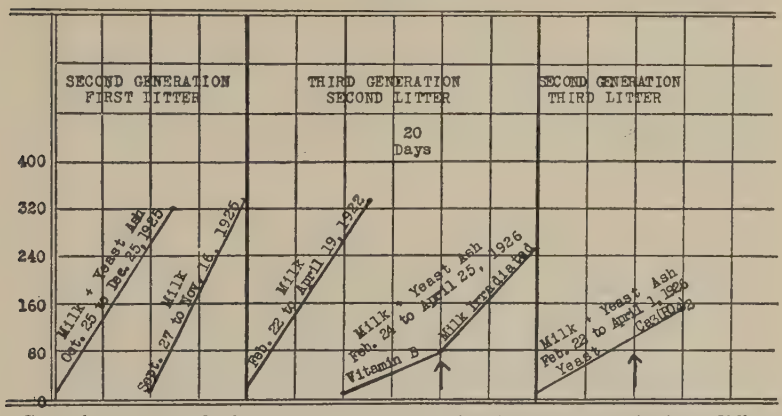
[2] Average percentage of sunshine in the reports of the United States Weather Bureau for Iowa City was:

	January	February	March	April
1922	66.4	62.8	44.8	47.7
1925	59.0	40.4	67.4	59.3
1926	48.2	44.9	44.6	64.3

[3] Steenboch, H., Hart, E. B., Holport, C. A., and Black, Archie, *J. Biol. Chem.*, 1925, lxiv, 441; Hess, A. F., *J. Am. Med. Assn.*, 1925, lxxxiv, 1910; Co well, S. J., *Brit. Med. J.*, 1925, i, 594.

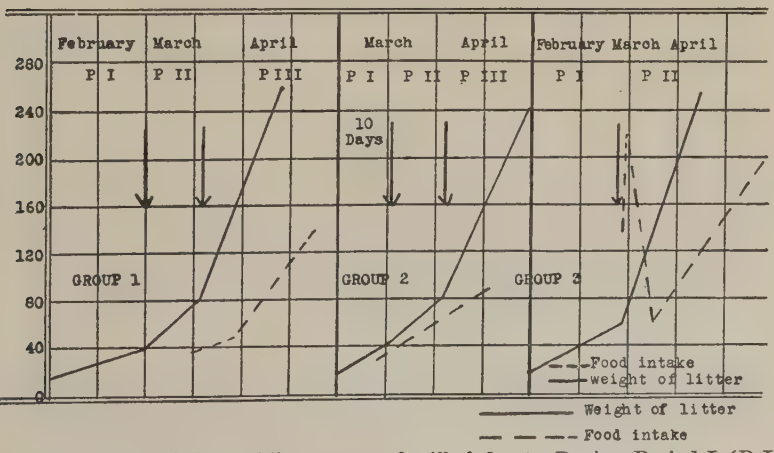
Steenboch and co-workers have observed differences in calcium balances in milking cows in different seasons.[4] It is possible that

CHART I.



Growth curves of the suckling young of milk-fed rats during different seasons, and during the same season, in different years. Neither the addition of a Vitamin B extract, yeast, nor calcium phosphate to the milk ration was as effective in promoting growth in the young, as the irradiation of winter milk with the ultra violet light from a quartz mercury vapor lamp.

CHART II.



Growth curves of the suckling young of milk-fed rats. During Period I (P I) the mother rats received milk plus the usual addition of iron and iodine. During Period II (P II), groups 1 and 2 received the cod liver oil additions. Groups 1 and 2 received irradiated milk in Period III (P III), while Group 3 received irradiated milk in Period II. The growth stimulation in Groups 1 and 2, therefore, was not the result of the cod liver oil, which in the two groups preceded the irradiated milk period.

[4] Hart, E. B., Steenboch, H., Elvehjem, C. A., and Scott, H., *J. Biol. Chem.*, 1926, xiii, 371.

our rats receiving the "winter" milk were suffering from rickets, and the irradiated milk brought about increased calcium and phosphorus balances. Macroscopic examination of the animals which died gave none of the characteristic symptoms of rickets, but since histologic examinations were not made, exact evidence is lacking. It would seem, however, that the very rapid growth of the animals on the irradiated milk, was more deep seated than just an increase in the anti-rachitic potency of the milk.

3190

Vitamin A content of fecal excretion of a breast fed and artificially fed infant. Preliminary report.

AMY L. DANIELS.

[*From the Department of Nutrition, Iowa Child Welfare Research Station, State University of Iowa, Iowa City, Iowa.*]

Rats fed on diets adequate in all other respects but low in the Vitamin A factor, develop, in the course of time, infection of the upper respiratory tract. The first symptoms of the dietary deficiency are snuffles, loss of appetite, and failure to gain. These animals invariably die unless appropriate dietary treatment is introduced fairly early in the course of the infection. Macroscopic examination of the various organs of these animals, with the exception of the aural and nasal passages, which have been found filled with pus, shows nothing consistently abnormal. Occasionally the lungs are infected, often the intestines are filled with gas and greatly distended. Death in these animals seems to be the result of an infection superimposed on tissues which have been altered by the dietary deficiency.

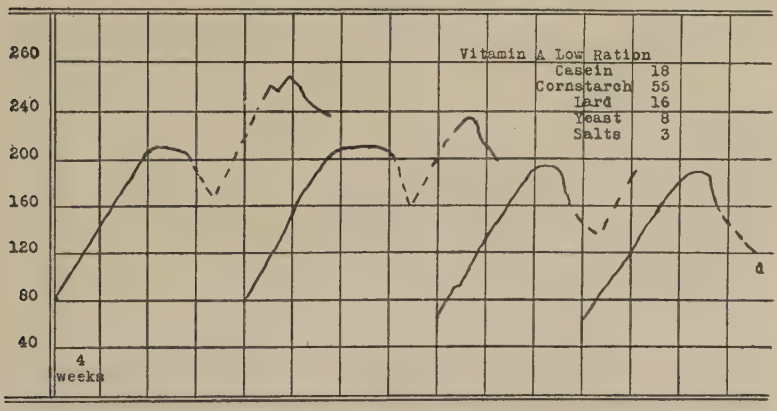
The excessive reaction to infection of the upper respiratory tract (nasal and aural passages) of the artificially fed baby and the low incidents of such infection amongst breast fed babies, suggests that in the former case as in the rats, we are dealing with an infection superimposed on tissues which have been altered by a dietary deficiency.

The possibility of the occlusion of Vitamin A in the calcium soap excreted by the artificially fed baby, and the consequent failure to absorb enough to meet the physiological needs, was

suggested by Daniels and Armstrong in 1923.[1] Acting upon this suggestion a comparative study of the Vitamin A excretion by way of the gut, in an artificially fed and in a breast fed infant, has been made.

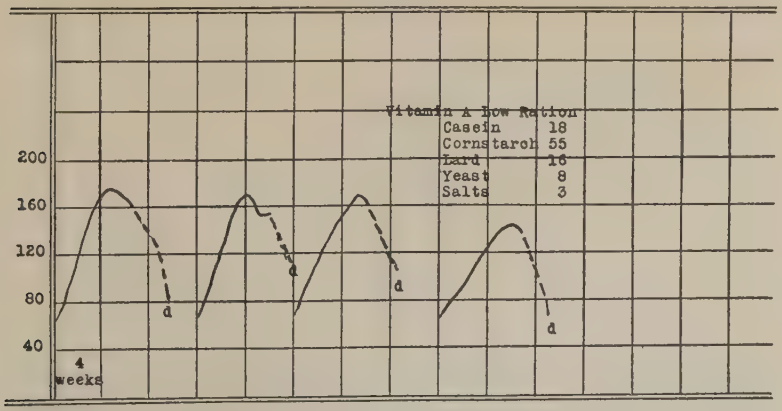
In the investigation, rats, at the weaning period were placed on purified diets, low but not wholly lacking in Vitamin A. When the weights of these animals became stationary and they had developed the characteristic symptoms of the infection of the

CHART I.



Growth curves of animals on Vitamin A low diets. The broken line indicates period of addition of ether extract, of artificially fed infant's stool.

CHART II.



Growth curves of animals on Vitamin A low diets. The broken line indicates period of addition of ether extract of breast fed infant's stool.

[1] Daniels, A. L., Armstrong, M. E., and Hutton, M. K., *J. Am. Med. Assn.*, 1923, lxxxi, 828.

upper respiratory tract, the ether extract of a three weeks fecal excretion of a seven months old baby, who was receiving a milk, dextri-maltose feeding mixture, was incorporated in the "A" low ration and was fed to a group of four animals over a corresponding period of time. To the Vitamin A low diet of another group of rats was added the ether extract of the three weeks fecal excretion of a six months old baby, who was at the same time receiving, in addition to the breast milk, one teaspoonful of cod liver oil and one ounce of orange juice daily.

With one exception, the animals receiving the ether extract of the artificially fed baby's stool gained in weight and apparently recovered from the infection; whereas, those receiving the extract of the breast fed baby's stool all died. From these results it would seem that the Vitamin A in the food of the breast fed baby is more completely absorbed than is the Vitamin A content of the food of the artificially fed baby.

3191

The colorimetric estimation of the hydrogen ion concentration of urine.

EDWARD MUNTWYLER, E. R. NORRIS and V. C. MYERS.

[*From the Biochemical Laboratory, State University of Iowa, Iowa City, Iowa.*]

A year ago a simple technique of estimating the pH of urine was suggested,[1] in which application was made of the bicolorimeter and the phthalein dyes, phenol red, brom cresol purple and brom cresol green. At that time we concluded that as far as the matching of colors went, the method had a probable error of pH $\pm$ 0.02 to 0.04, but we realized that the factors of temperature and dilution must exert some influence on the true pH. Obviously one desires to know the pH of the undiluted urine at body temperature. During the past year we have been trying to ascertain how far colorimetric determinations on diluted urine at room temperature differ from electrometric determinations on

[1] Myers, V. C., and Booher, L. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 511.

undiluted urine at 38°. In connection with an acid-base balance study we felt that we should know just how far the present colorimetric determinations might deviate from the true pH values and if a correction factor might be applied which would hold for all urines.

Since our preliminary report was made and the present study begun, Hastings, Sendroy and Robson[2] have reported a study of this question. They carry out their determinations at 38° diluting the urine 1-5. They state that the dilution error is the important error and amounts to about 0.1 pH, which must be subtracted from the colorimetric result. They believe that with this correction their results fall within 0.1 of the actual pH.

In routine work it is quite troublesome to carry out colorimetric estimation at 38°. In the present study it has been found that the factor necessary to correct for the determination being made on the diluted urine at room temperature amounts to roughly 0.2 pH, about half of which is a temperature correction and half a dilution (salt and buffer) correction. The latter factor, however, is by no means constant.

For eight normal urines of similar sodium chloride and phosphate content the average difference between the colorimetric reading determined with brom cresol purple at room temperature on urine diluted 1-5 and the electrometric value of the undiluted specimen at 38° had an average value of 0.2 pH. However, even when the pH of the urine samples were close together the corrections varied from pH 0.14 to 0.28.

Sörenson's phosphate solutions having an 0.067 M concentration give very good agreement when determined colorimetrically and electrometrically at the same temperature. This is true whether either brom cresol purple or phenol red is used as the indicator. However, with concentrations of phosphate greater than 0.067 M the colorimetric values become greater than the electrometric values, while with concentrations less than 0.067 the electrometric values become greater. Lepper and Martin[3] have already pointed this out for phenol red.

Solutions in which the sodium chloride content is varied and the phosphate concentration kept constant give colorimetric pH

[2] Hastings, A. B., Sendroy, J., and Robson, W., *J. Biol. Chem.*, 1925, lxv, 381.

[3] Lepper, E. H., and Martin, C. J., *Biochem. J.*, 1926, xx, 45.

values which vary from electrometric values when determined at the same temperature. At concentrations of sodium chloride greater than 0.06 M the colorimetric values become greater and increase over the electrometric values as the molarity of the sodium chloride increases. For example, using either brom cresol purple or phenol red indicators, if we plot the difference between the colorimetric and electrometric values as ordinates and the molarity of the salt in a constant 0.02 M phosphate solution as abscissae, the curve crosses the 0 at about 0.06 M NaCl and increases to the point where at 0.5 M NaCl the colorimetric value is 0.2 pH greater than the electrometric value.

In attempting to obtain relations between the difference of the electrometric pH at 38° undiluted and the colorimetric pH of the 5 fold diluted specimen at room temperature, and the salt content, using pure solutions of urea, phosphate, sodium bicarbonate and sodium chloride in concentrations found in the urine, identical solutions from day to day would not give the same results. In studying the reason for this it was found that the pH of the distilled water used for dilution has considerable effect on the dilution curve. A solution with a high pH will have a different dilution curve than a solution of a low pH when diluted with the same water having a low pH.

It thus hardly seems possible that we can have a single constant which will hold accurately for all urine samples, using the method now employed for the pH determinations. Work is being continued in an attempt to standardize the colorimetric method.

3192

The influence of the ingestion of methylated xanthines on the excretion of uric acid.

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In 1916 Benedict[1] reported a single experiment in which the ingestion of caffeine lead to an increased output of uric acid as determined by the then new Benedict-Hitchcock colorimetric

[1] Benedict, S. R., *J. Lab. and Clin. Med.*, 1916, ii, 1.

method. A year later Mendel and Wardell[2] studied this question with the same method, employing not only caffeine, but also coffee and tea. They observed an unmistakable increase in the uric acid output in all cases. The statements in the older literature are quite contradictory. Many texts still state that the ingestion of methylated xanthines is without effect on the excretion of uric acid. For this reason it seemed desirable to again study this problem.

In the present studies metabolism experiments have been carried out with the three common methylated xanthines, viz., caffeine, theobromine and theophylline, employing several methods for the estimation of uric acid. In all experiments the subjects were placed on purine-free constant diets until the uric acid excretion reached its endogenous level. The methylated purine to be studied was then added to the diet for a period of three or four days and finally the purine-free diet was continued for three days longer. Estimation of uric acid excretion were always made by the Benedict-Franke colorimetric method; in certain cases they were made also by the Benedict-Hitchcock colorimetric method and by the Krüger-Schmidt precipitation method.

With two subjects the excretion of uric acid was markedly increased throughout the period of caffeine ingestion, but when the caffeine was discontinued, the uric acid fell back to its endogenous level within 48 hours. With two other subjects the excretion of uric acid was markedly increased on the first day of caffeine ingestion, then gradually decreased until on the last day of caffeine ingestion the uric acid was back to its endogenous level, where it remained practically constant throughout the final period. Estimations made by the Benedict-Hitchcock and Benedict-Franke methods always gave comparable results. When the Krüger-Schmidt method was used, it was found that the increase was in no way comparable to the increase as estimated by the Benedict-Franke method.

The possibility that this discrepancy in the results obtained by the two methods might be due to some product of caffeine metabolism other than uric acid led to the study of the color forming power of certain methylated uric acids which were kindly furnished by Professor Biltz of the University of Breslau. Of the three compounds studied, 7-methyl uric acid and 3-7-dimethyl

[2] Mendel, L. B., and Wardell, E. L., *J. Am. Med. Assn.*, 1917, lxviii, 1805.

uric acid gave only a faint trace of color with either the Benedict-Hitchcock or the Benedict-Franke procedure. The third compound, 1-3-dimethyl uric acid, gave no color at all with the Benedict-Hitchcock procedure but with the Benedict-Franke procedure its color forming power was practically as great as that of uric acid itself.

In experiments with theobromine the ingestion of 2 gm. of theobromine per day for a period of four days caused no increase in the excretion of uric acid as estimated by the Benedict-Franke method, although the greatly increased excretion of purine bases gave evidence of excellent absorption. The fact that neither 7-methyl- or 3-7-methyl uric acid yield any color with the Benedict-Franke reagent is of some interest in this connection.

A single experiment with theophylline (euphyllin) showed a marked increase in uric acid excretion. In the first part of the euphyllin period, the uric acid as estimated by the Benedict-Franke method was much greater than when estimated by the Benedict-Hitchcock procedure. During the euphyllin period the Benedict-Hitchcock values gradually rose and the Benedict-Franke values fell until they were practically equal at a point definitely above the endogenous level. The fact that 1-3-dimethyl uric acid reacts with the Benedict-Franke reagent, may have some bearing on the present findings.

3193

The colorimetric estimation of methylguanidine in biological fluids. (Preliminary report).

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Methods for the estimation of guanidine and its methyl derivatives have been subject to considerable criticism. A study of the color reaction for guanidines recently described by Marston[1] was undertaken in order to determine its biochemical applicability. Although Marston specifically states that his reagent will

[1] Marston, H. R., *Aust. J. Exp. Biol. and Med. Sci.*, 1924, i, 99.

keep indefinitely, in our hands deterioration and precipitation invariably set in shortly after mixing the three ingredients, sodium nitroprusside, potassium ferrocyanide and sodium hydroxide.

A reagent has been developed, which, although it contains the same ingredients as employed by Marston, has the advantage that it keeps well and gives within five minutes a full color development which does not fade or become turbid for more than an hour, thus allowing ample time for color comparison.

The reagent we now employ is prepared as follows: 6 gm. sodium nitroprusside and 8.5 gm. potassium ferrocyanide are dissolved in water and made up to 100 cc. About 15 to 20 minutes before using, one volume of this solution is mixed with one volume of 10 per cent sodium hydroxide and two volumes of 3 per cent hydrogen peroxide. It has been found convenient to add 1 cc. of the prepared reagent to 4 cc. of the unknown guanidine solution and compare in a colorimeter with standards similarly prepared. With this technique quantities of guanidine bases as small as 0.2 mg. may be estimated.

Creatine and creatinine give a faint coloration with the reagent but do not interfere materially in the estimations. Uric acid and ammonia interfere by hindering the color development with the bases. Urea produces about one tenth of the color given by guanidine, a fact not mentioned by Marston. Methyl urea, β-methyl hydantoin, β-methyl hydantoic acid and glucose yield no color, while ethyl alcohol gives a very faint reaction.

In applying the color reaction to the colorimetric estimation of methylguanidine in biological fluids we attempted to use permutit to remove ammonia as suggested by Marston.[2] However, our findings show that permutit removes guanidine, methyl guanidine, and *as* -dimethyl-guanidine from aqueous solution but not quantitatively.

At present we are employing the following technique for the estimation of methylguanidine in blood: 10 cc. of blood are treated with a suitable amount of urease and buffer phosphate solution, incubated for half an hour at 50° and then precipitated with the Folin-Wu method in the usual manner. 1 cc. of saturated sodium carbonate is added to the filtrate and this evaporated to dryness. The residue is then extracted with three

[2] Marston, H. R., *Aust. J. Exp. Biol. and Med. Sci.*, 1925, ii, 57.

successive 10 cc. portions of absolute alcohol, filtered, evaporated to dryness, taken up in 4 cc. of water and color developed as described. Readings can best be made at the end of 10 minutes.

We have had the opportunity of applying the method to the blood and pleural fluid of a case of chronic nephritis, with a blood creatinine of 24 mg. and a urea N of 162 mg. per 100 cc. The color reaction indicated 10 mg. of methylguanidine in the former and 15 mg. in the latter. In a case of hypertension without nitrogen retention the test indicated 10 mg. per 100 cc. Tests on the blood of normal subjects indicate that, if methylguanidine is present, the amount is less than 0.2 mg. per 100 cc.

Bearing in mind that this color reaction is not entirely specific for guanidine bases, it would appear that either guanidine bases are present in estimable quantity in nephritic blood or some unknown substance is present which gives the reaction. The results could not have been due to urea or creatinine since no appreciable interference was noted in controls which contained comparable quantities of both substances.

3194

A new adsorbent for creatinine.

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The use of kaolin in removing creatinine from dilute solution is disadvantageous in that the adsorbed creatinine cannot be released again for identification. An adsorbing agent which can be decomposed under conditions that will not destroy creatinine therefore appears desirable.

If one adds to 10 cc. of the strongest creatinine standard for blood determinations (1 mg. of creatinine in 100 cc. of saturated picric acid), 0.1 cc. of 9.5 per cent potassium chloride, and 1 cc. of 10 per cent phosphotungstic acid, a finely divided yellow precipitate forms, which settles out very slowly, but can be centrifuged out quickly. The supernatant liquid is decanted, and the

precipitate suspended in 10 cc. of saturated picric acid. On adding 1 cc. of 10 per cent sodium hydroxide, the precipitate dissolves and the color developed matches that of 10 cc. of the same standard, made alkaline directly with the same amount of sodium hydroxide. Controls with the potassium precipitate in absence of creatinine are negative.

Complete adsorption of creatinine is practically coincident with appearance of the precipitate. Supersaturation is occasionally encountered. If the precipitate fails to appear, the difficulty is overcome, when working with larger amounts, by adding a drop of potassium chloride solution after the phosphotungstic acid. The precipitate is more difficult to centrifuge out if all of the potassium chloride is added after the phosphotungstic acid.

The precipitate obtained from a liter of saturated picric acid containing 10 mg. of creatinine, on adding 10 cc. of 9.5 per cent potassium chloride, and 100 cc. of 10 per cent phosphotungstic acid, can be suspended in the centrifuge tube in which it has been collected, in 30 cc. of normal sulfuric acid. On diluting the mixture to about 50 cc. and shaking with ether, an emulsion forms, which separates on a moment's centrifuging into three sharp layers, predominantly ether, sulfuric acid, and phosphotungstic acid solutions. The latter contains undecomposed precipitate at first. The ether layer is siphoned off, and the shaking repeated with eight or ten fresh portions of ether until all of the picric acid has been removed. The middle layer is then siphoned off. It contains over 80 per cent of the creatinine. Sulfuric and a trace of phosphotungstic acid are removed with basic lead acetate solution. The excess of lead is removed with hydrogen sulfide, and the filtrate evaporated. A residue of potassium acetate and creatinine remains. This can be transferred to a tube with 10 cc. of absolute alcohol in small portions, and most of the potassium is precipitated by additions of concentrated hydrochloric acid until a drop removed with a stirring rod and placed on wet congo paper gives a blue color. After centrifuging and decanting, the alcoholic solution is evaporated, and the residue transferred to a small test tube with portions of water totalling 10 cubic centimeters. Colorimetric analysis shows presence of about 7 mg. of the original 10 mg. of creatinine. Dry picric acid, 130 mg., is now added, and the tube heated. On cooling, potassium creatinine picrate crystallizes out, only 0.4 mg. creatinine remaining in solution. After centrifuging and decanting the

liquid, the picrate can be recrystallized in the same tube from 3 cc. of water, washed with a similar quantity of absolute alcohol, then with ether, and dried. It is tested for purity by weighing 10 mg. accurately, and determining the creatinine content colorimetrically.

If phosphotungstic acid in the above amounts is added to saturated picric acid filtrates from blood or pleural transudate, a precipitate forms, which, if the blood is not oxalated with potassium oxalate, is not due to potassium ion. A large amount of the substance giving the Jaffé reaction is adsorbed. The residue, on evaporation after hydrogen sulfide treatment in the above scheme, can be taken up directly in water, and saturated with picric acid in the manner described. On cooling this picric acid solution of the residues from 400 cc. portions of postmortem blood from two nephritics, no precipitate formed. The same finding held for fluid from the pleural cavity, exudate in one case and transudate in the other. But on addition of potassium chloride, creatinine potassium picrate crystallized out of all of the picric acid solutions easily, and was purified and identified as above. The samples contained 18.2 to 18.7 per cent creatinine (theory 18.5), and a solution of the same lost its creatinine on treatment with kaolin.

Unfortunately the potassium precipitate adsorbs creatine also. When amounts of creatine varying from 20 to 100 mg. (as creatinine) were added to a liter of saturated picric acid containing 10 mg. of creatinine, the additional creatinine in the final residue increased steadily, in each case corresponding to about 22 per cent of the creatine present. Whether the precipitate produced on adding phosphotungstic acid to picric acid filtrates from the fluids examined adsorbs creatine cannot be decided at present. In the decomposition, the sulfuric acid layers, according to colorimetric methods, contained far too little creatine to account for the creatinine potassium picrate isolated, and the substance responsible disappeared in the basic lead acetate precipitation.

The "creatinine" content of both of the bloods studied was essentially the same as on repeated examination before death. The fluid from the pleural cavity gave values almost identical with those of the blood samples, which contained 5.9 and 24.0 mg. respectively, but fully half of the "creatinine" of the pleural transudate and exudate was removable by kaolin. Further study

of this point may be of interest, both in connection with the question of presence of creatinine in blood, and with the site of formation.

3195

A study of interrupted duodenal obstruction in the rabbit.

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The obstruction was produced, 20 cm. below the pylorus, by means of a ligature tied over an elastic compressor on the outside of the abdominal wall. Such an obstruction causes almost no trauma to the intestine, and can be released at any time desired without employing a second anesthetic and laparotomy, with their complicating effects.

If the obstruction at this level is not released, the period of survival averages seventeen hours. The variations from this average time are within three hours.

If the obstruction is released after a duration of fifteen hours or less, the animal survives. If, however, the obstruction is released after a duration of sixteen hours or more, the animal does not survive.

The sharp line of demarcation between the duration which is fatal, and that which is followed by recovery is quite striking. Also, the recovery of the animals from an obstruction of twelve to fifteen hours duration was remarkably rapid. Even though such animals before release of the obstruction gave evidence of being in a very serious condition, a striking degree of recovery is shown within one to three hours following the release of obstruction. If the animal's condition was due to absorption of a highly toxic substance from the obstructed content, such rapid recovery would hardly be expected. If the condition were due, however, to depletion of chloride[1] or the loss of fixed base,[2] the rapid

[1] Hayden, R. L., and Orr, T. G., *J. Exp. Med.*, 1923, xxxvii, 365.

[2] Gamble, J. L., and McIver, Monroe A., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 365.

recovery could be the result of rapid reabsorption of these substances which accumulate in the fluid distending the stomach, since the non-operative release of obstruction allows the obstructed content to move rapidly on to the lower parts of the canal where absorption is rapid.

To test this point, further experiments were performed in which the obstruction was released after fourteen hours, but just prior to release, the stomach was emptied of its accumulated fluid and washed with distilled water. Distilled water was left in the stomach so that loss of water was not a factor in the result. None of these animals survived. Autopsy showed that the release had been complete and that no performation or peritonitis or other observable complication existed.

Since these animals, in which the stomach was evacuated prior to release of obstruction, succumbed to a duration of obstruction which had been shown in the earlier series of experiments to be a non-fatal duration, the experiments indicate that the cause of early death of rabbits with high intestinal obstruction is not due to dehydration, nor absorption of a toxic substance, nor extreme gastric dilatation, but to a loss in the gastric contents of something essential to the animal's recovery. This loss is likely the chloride and sodium as held by the writers referred to above.

3196

Influence of continued administration of morphine and of withdrawal on contraction of small intestines of dogs.

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Two experiments were carried out on dogs with Thiry-Vella fistulae of ileum. The operations for formation of the fistulae were performed several weeks before the experiments were started. Graphic records of the intestinal contractions were made by introducing into the fistula a sausage-shaped rubber balloon fastened on a catheter and filled with water; the catheter was connected with a Brodie bellows-recorder which made the tracing on

a slowly moving kymograph. Records were made in this way without anesthesia or operative procedure or discomfort to the animal.

Experiment 1. Ascending doses of morphine sulphate: A female collie dog, weighing 21 kg. received daily hypodermic injections for 71 days and during this time the dose was increased from 1 mg. per kg. to 25 mg. per kg. There was some constipation and loss of appetite but the dog remained in good condition, losing only one kg. in weight. Narcosis and vomiting disappeared after the fourth week, but salivation continued throughout the experiment. The dog became shy and restless as the experiment progressed.

Graphic records of the effect of the injections of morphine on the intestinal contractions were made at intervals of two to three days throughout the experiment. There was no change in the character of the reaction at any time. We have reported in a recent paper[1] that hypodermic injections of morphine in dogs greatly increase the muscular activity of the intestine. In this experiment each injection produced the usual reaction; marked increase in tone and in amplitude and frequency of the contractions. From time to time small doses (0.15 mg. per kg.) were injected before the regular daily dose, and these invariably produced stimulation of the intestinal contractions. The reaction following a small dose was as great at the end of the experiment, when the daily dose was 25 mg. per kg., as it had been at the beginning.

The administration of morphine was discontinued entirely on the 72nd day of the experiment. During the first 10 days of the withdrawal period there was a marked increase in the frequency and amplitude of the intestinal contractions. At this time the dog had diarrhea. The animal slept more than usual during the early part of the withdrawal and for a time was hard to control while the tracings were being made.

Experiment 2. Daily repetition of a small dose of morphine sulphate: A female collie dog, weighing 23 kg., received daily injections of 0.1 mg. per kg. for six weeks. Records of intestinal contractions were made every second or third day. This small dose continued to produce stimulation of the intestinal contractions throughout the experiment, the reaction being as marked

[1] Plant, O. H., and Miller, G. H., *J. Pharm. and Exp. Therap.*, (in press).

after 6 weeks as when the injections were started. Narcosis, nausea and vomiting disappeared after 2 weeks, and from that time to the end of the experiment the dog appeared normal in every way. The loss in weight amounted to 2.5 kg., but appetite remained good. Administration was stopped on the 42nd day. No changes could be observed in the intestinal contractions or in behavior during the withdrawal period.

These results show that tolerance is not developed to the stimulating effect of morphine on the intestinal contractions. They thus parallel the observations of van Egmond[2] that the cardiac vagus center does not become tolerant to morphine in dogs. Further, they indicate that the increased destruction of morphine in the tissues, which was demonstrated by Faust,[3] is not the only factor concerned in the development of morphine tolerance.

[2] van Egmond, A. A. J., *Arch. f. exp. Path. u. Pharmak.*, 1911, lxv, 197.
[3] Faust, E. S., *Arch. f. exp. Path. u. Pharmak.*, 1900, xliv, 217.

Southern Branch

Tulane University, May 12, 1926.

3197

The fate of two synthetic amino acids.

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The mode of catabolism of the diamino acids is obscure. It has been shown that ornithine is a sugar former in the diabetic organism, while lysine is not. It appears that alpha-amino acids are frequently broken down through the stage of a fatty acid having one less carbon atom.[1] If ornithine and lysine took this route, they would form gamma-amino butyric and delta-amino valeric acids respectively. As it seemed of interest to determine the fate of these substances in the diabetic animal, they were synthesized and administered to phlorhizinized dogs. The results would indicate that delta-amino valeric acid fails to form extra glucose while gamma-amino butyric acid is a sugar former. The possibility is therefore to be considered that one of the paths of catabolism of the diamino acids is through the stage of mono-amino acid with one less carbon atom and with the amino group in the terminal position.

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[1] Dakin, H. D., "Oxidations and Reductions in the Animal Body," 2nd ed., 1922, Longmans, Green & Co., London.

3198

Histological changes produced by inversion of nipple flaps of mammary gland of the rabbit.*

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Inverted and bleeding nipples and chronic inflammatory lesions of the human breast are frequently noted as forerunners of cancer of this structure. These conditions often present the aspect of the "indifferent vorstadium" described by Ribbert[1] or the picture commonly alluded to as the so-called precancerous stage.

The experiments herein noted were performed with the view of ascertaining what results would follow the inversion of nipple flaps in the mammary gland of the female rabbit.

Eight female rabbits purchased in the open market were employed and their histories and ages are therefore not known. Two of these rabbits were lactating and one other was not full grown. Twenty-eight operations were performed. Eighteen different nipple flaps were inverted and ten of the nodular masses thus produced were bisected and reinverted in other areas. The operations, as a whole, consisted in cutting a rectangular flap in which the nipple was included and the basal attachment of the flap was not disturbed, thus maintaining the circulation from that source. The depth of the flap extended down to the areolar tissue, thus including the skin and gland structure beneath. It may be mentioned that in the quiescent breast, the gland of the rabbit is rather rudimentary in its gross aspect; where lactation is present the gland is well developed and it was sectioned completely through in order to procure the flap. The tip of the nipple was clipped off and the stump cross sectioned and scraped. The underlying gland was also scraped and partially sectioned. The flap thus prepared was inverted with the nipple downward and then drawn beneath the overlying structures by means of a retaining suture. When the inverted flap was in the vicinity of the

* Aided by a grant from the B. M. Harrod Cancer Research Fund.

1 Ribbert, "Geschwulstlehre," Boun, 1904 or *Aertz. Sach. Zeit.*, 1898, 389, cited in "Neoplastic Diseases," Jas. Ewing, 1922, p. 993.

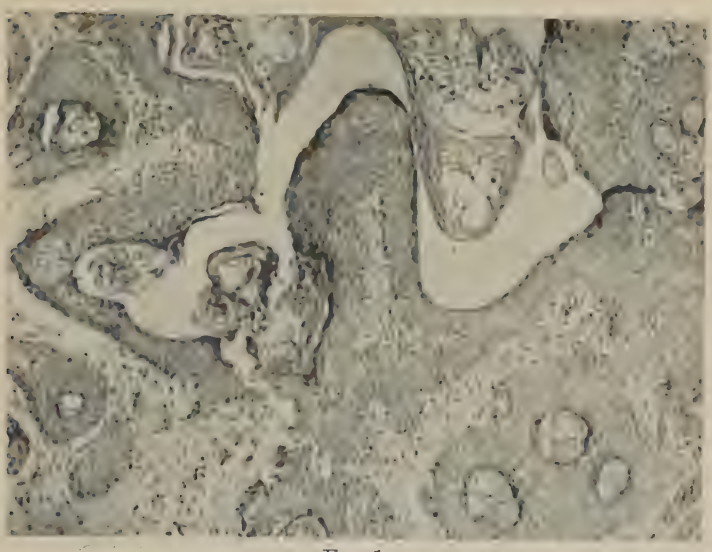

FIG. 1.

Papillary protrusions and epithelial cell multiplication about hair shafts and occurring in skin epithelium of inverted flap.

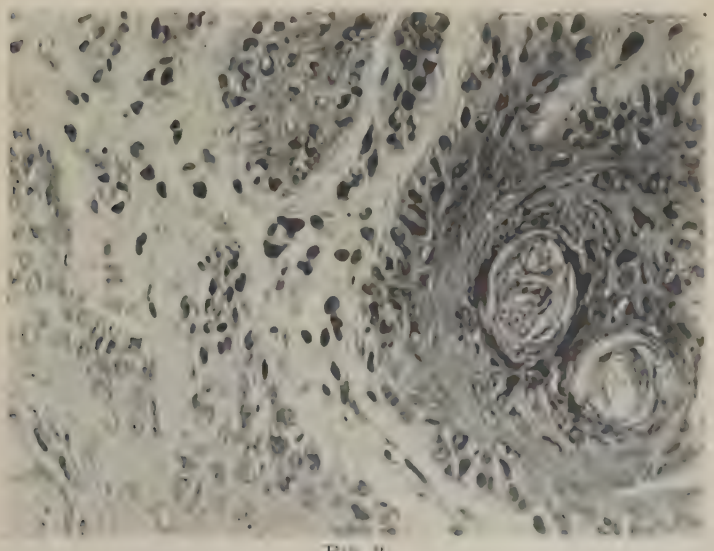

FIG. 2.

High power area corresponding to Fig. 1 showing rapidly growing epithelium with mitotic figures.

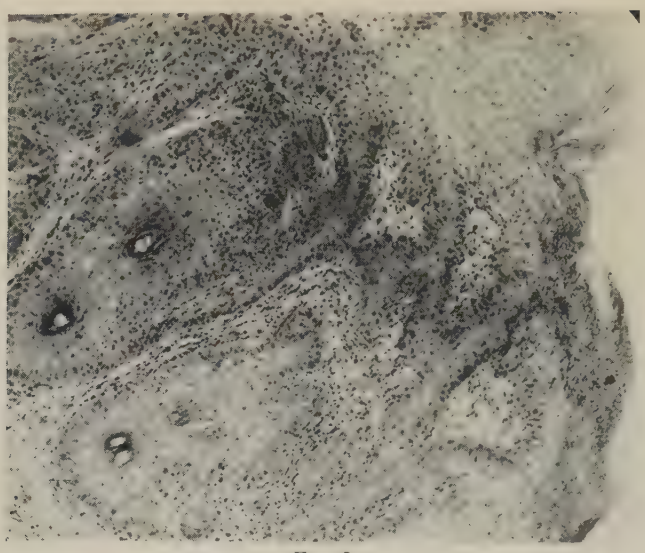

FIG. 3.

Section of inverted flap after 4 weeks standing, presenting extensive proliferation of squamous epithelium of hair shafts and surface with downward extension of skin epithelium, simulating epithelioma.

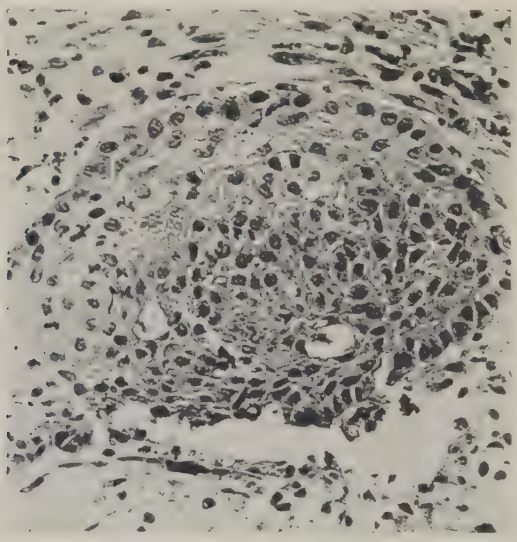

FIG. 4.

Island of prickle or spine cells located deep beneath the surface epithelium. Many mitotic figures are present.

FIG. 5.

An isolated strand of skin epithelium demonstrating organization by fibroblastic cells with the presence of scattered polymorphonuclear leucocytes and a foreign body giant cell; all of which are processes of extinction of the growing epithelium.

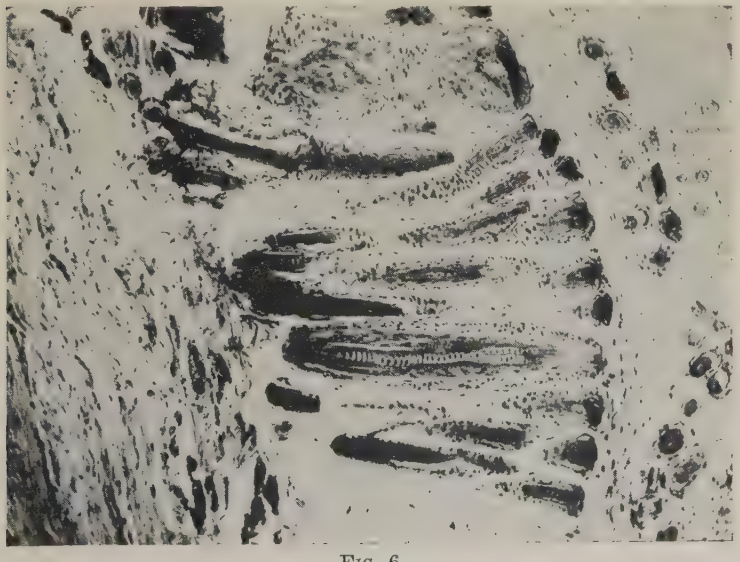

FIG. 6.

Hair growing downward from the inverted flap. This growth often attained an inch or more in length.

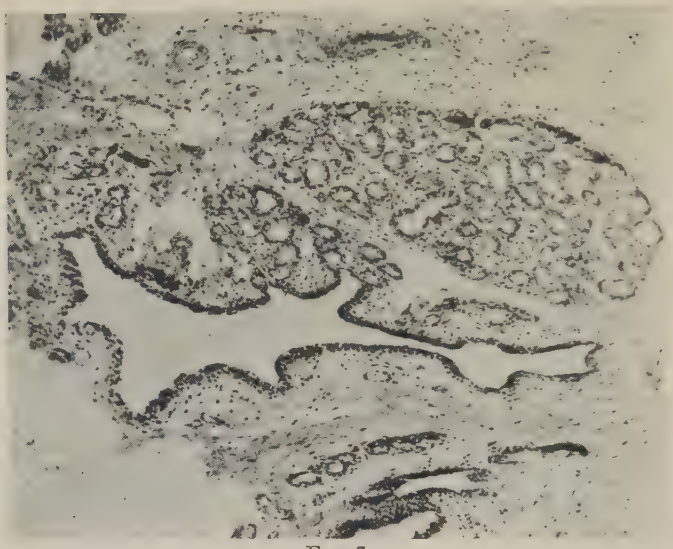

FIG. 7.

Inverted mammary gland structure persistent and well preserved after one month.

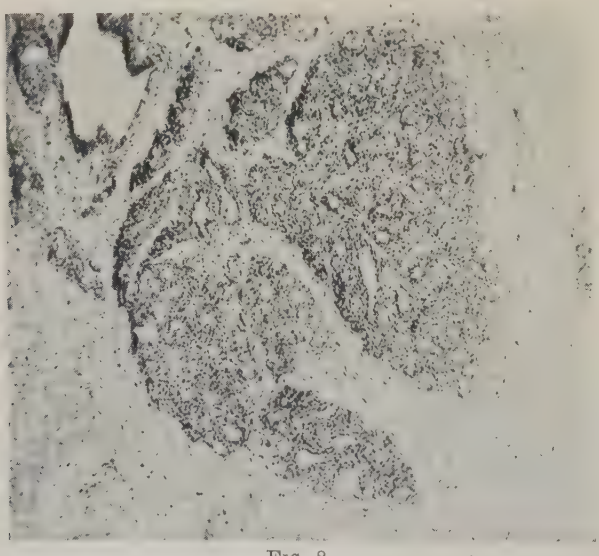

FIG. 8.

Ducts of mammary gland showing aberrant growth of epithelium after inversion for 6 weeks.

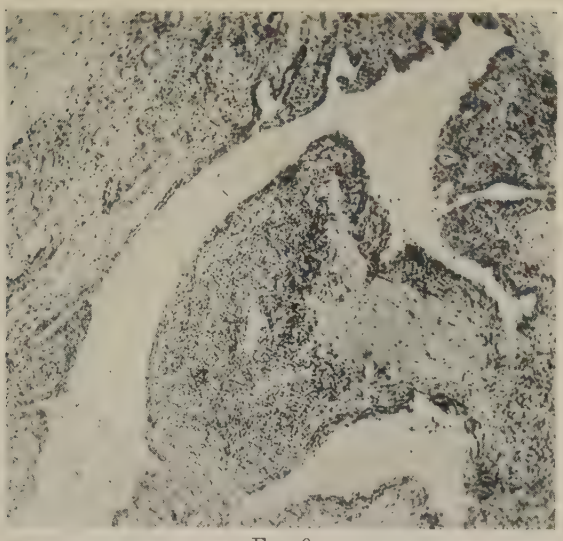

FIG. 9.

Intracanalicular papillary protrusion covered with multiple layers of epithelium occurring in the inverted mammary gland flap.

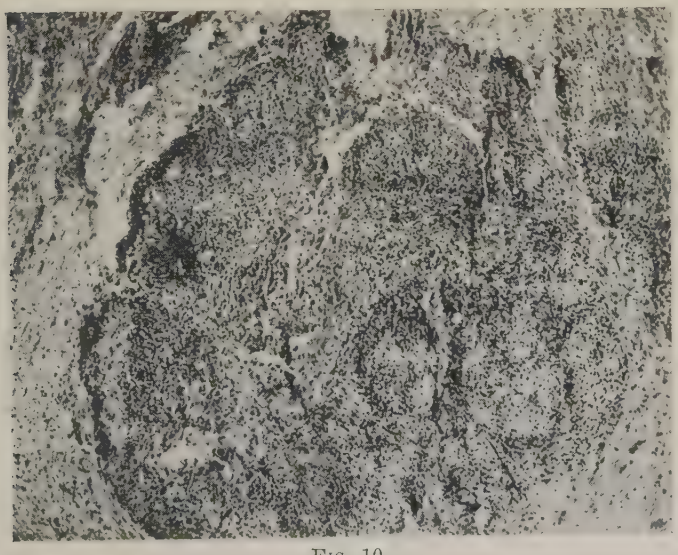

FIG. 10.

Massive irregular growth of epithelium derived from the gland structure, simulating carcinoma in aspect. Flap inversion is of eight weeks standing.

pectoral muscle it was imbedded beneath this structure and retained there by a suture. The gaping wound remaining was sutured lightly together so as not to severely embarrass the circulation at the base and a sterile dressing was applied. The operations and observations have extended over a period of seven months.

As a result of this operative procedure, reactionary nodules were formed in all instances. Acute inflammatory evidences were present primarily both grossly and microscopically about the inverted flap. The congestion and edema subsided after two weeks but nodulation persisted. Study was directed especially towards the action of the epithelial cells placed under these abnormal conditions. It is of interest to note that greater activity was observed on the part of the skin epithelium of the inverted flap than for that of the gland cells. This fact is probably due to the greater proliferative tendency of skin epithelium, and also because its misplacement was more abnormal than that of the gland cells. Portions of the nodules that were formed were removed at various periods of time such as after the first, second, third, fourth, fifth, sixth, seventh and eighth weeks. In the microscopic study of the sections it was found that the inverted gland epithelium persisted in duct formation for four or five weeks. In certain nodules the cells grew at random from the confines of the basement membrane and the tubular arrangement was lost or only poorly shown in certain areas. It is noteworthy, however, that these cells did not assume the distinctly modified aspect found in cancer cells, *i. e.*, their general morphology and tinctorial property adhered rather closely to the original cell type. In the instance of the epithelium of the skin and hair shafts, however, the cells were frequently of a more aberrant type. Often appearances analagous to those of the basal and spine-celled epitheliomata could be found. The epithelial cells of the hair shafts were especially active in their proliferation and mitosis could be noted. In some nodules continued growth of the hair shaft in an inverted manner was found. At times there were present cystic masses containing sebaceous matter and hair resembling in aspect a dermoid cyst. Enlargement of the adjacent lymphatic glands was frequently noted and at times specimens were removed for examination. They were found to present, microscopically, chronic proliferative adenitis, probably the results of absorption of cell products.

Of special note were the defensive methods presented by the host, preventing the continued aberrant growth of these misplaced epithelial cells. Primarily, the inflammatory cells, through their activity, produced degenerative changes in the flap cells, but of greater importance was the connective tissue response which seemed to form the main defensive barrier, not only surrounding and barricading the cell growth, and curtailing its blood supply, but actually invading and organizing the mass. The fibroblastic cells arose from all sides and appeared to actually crush out of existence the threatened continued epithelial growth. Occasionally, giant cells of the foreign body type appeared as destroying factors. The microphotographs illustrate a variety of the histological changes observed.

Although the nodules thus far studied appeared at times to thrive, and attained in part the appearance of malignant growth, the eventual outcome, as a whole, was degeneration and fibrosis. However, three animals with persistent nodules are still under observation.

The failure of formation of true epithelial neoplasms in these experiments appear attributable, not to the lack of impetus or inherent cell proclivity of the invading epithelium but rather to the inhibitory or restraining influences put into action by the defensive factors of the host. It is known that some species of animals demonstrate a natural resistance to epithelial neoplasm, especially for certain anatomical areas. It is not unlikely that such factors of resistance prevail to a great extent in the mammary gland of the rabbit.

3199

Evidence limiting the time of inception of intrauterine digital amputations.

HAROLD CUMMINS.

[*From the Department of Anatomy of the Tulane University School of Medicine, New Orleans, La.*]

Evidence is derived from epidermal ridge configurations, in four subjects admitting diagnosis of congenital amputations (as opposed to agenesia), which points to the existence of the affec-

tion probably prior to the eleventh week of gestation. Three of the cases are considered in another publication,[1] where they are recorded as numbers 20, 508, and 509; a fourth, a still-born infant presenting multiple digital amputations in varied degrees, is comparable to 509.

It has been shown[1] that the alignment of epidermal ridges, hence their fashioning of patterns and of patternless series of ridges, is accomplished through the medium of growth forces obtaining in early fetal development. Such forces in growth vary locally, in accordance with the irregular molding of palmar and plantar reliefs. The influence on alignment hardly can be effective after ridges are initially elaborated (eleventh week), although it is possible that the regulation through growth may manifest its effect before the ridge anlagen actually appear. There is no evidence to show that a ridge arrangement, once effected, can be altered, barring, of course, its participation in the generalized increase of size. With this premise of permanence, coupled with the demonstrated genetic relation existing between the form of a part and the character of its ridge configuration, it is warranted to assume that a congenitally defective hand or foot will display configurations conforming to its particularized molding if the abnormality existed during the critical period of ridge determination. Such conformity is invariable in abnormalities which are initially present in the member (such as syndactylism, ectrodactylism, etc.). The conformity occurs also in the examples of amputation here reported, to the extent that the configurations are individually unique, being so far modified from the normal. This fact leads to the conclusion that the amputations had progressed far enough, by the time of ridge determination, to have affected the form of the involved parts.

Detailed description and figures are presented in the cited reference, where, it may be explained, the cases are utilized as illustrations of the faithfulness with which configurations are accommodated to contours.

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[1] Cummins, H., *Am. J. Anat.* (in press).

3200

A slide test for the diagnosis of syphilis.

H. W. BUTLER.

[*From the Department of Medicine of the Tulane University School of Medicine, New Orleans, La.*]

A simple slide test has been developed which seems to possess certain advantages over previously described serum tests. The stock antigen is stable. Inactivation of serum is unnecessary. The time required to make the test is two minutes. No special laboratory equipment or facilities are required.

Antigen preparation: Fresh veal hearts are selected, and the superficial fat removed. The muscle is ground in a sausage grinder, and spread on paper and dried by an electric fan. After it is completely dried, it is powdered in a mortar and extracted with ether. Four hundred cc. of ether are used to each 100 gm. of powdered heart and allowed to act for ten minutes, shaking frequently. The ether is filtered off and 300 cc. are again added to the heart and treated in the same manner. The heart muscle is again treated with 300 cc. of ether, and again a third time, the ether in each instance being filtered and discarded. The heart muscle is now dried free from ether, and for each gram of muscle, 5 cc. of 95 per cent alcohol is added and maceration is allowed to continue for three days at room temperature, after which the alcohol is filtered off and made up to the original volume with 95 per cent alcohol. This constitutes the defatted alcohol heart extract for the antigen. Six decigrams of cholesterol and 3 cc. of glacial acetic acid are added for each 100 cc. of the alcoholic extract. This is filtered after solution is effected and constitutes the finished special antigen for this test. This antigen seems to be stable for at least several weeks.

Technic: One cc. of antigen is measured into a test tube and 2 cc. of distilled water into a second tube (normal saline can be used, but the solution is unstable). Mixing is effected by pouring the solution from one tube to the other at least six times.

Two drops of serum are placed upon a clean slide about the junction of the middle and outer third. With a pipette, used only for the antigen dilution, three drops of the dilution are placed upon the slide near, but not into the serum. These are mixed

on the slide thoroughly with a toothpick, and the slide slowly rocked for two minutes. If the serum is positive, a characteristic granular precipitate which can be easily seen, develops during the rocking process. If negative, no specific precipitate forms within the two minute time limit.

3201

The nature of the toxic principle of the scarlet fever streptococcus for rabbits.

CHARLES W. DUVAL and R. J. HIBBARD.

[*From the Department of Pathology of the Tulane University School of Medicine, New Orleans, La.*]

In the present communication we wish to report the results obtained in an effort to produce toxic effects in animals with the *streptococcus scarlatinae* and to briefly discuss the toxemia and exanthem of the disease in the light of these experiments. The work was undertaken because of our previous failure to induce acute toxic nephritis in the rabbit either with large doses of viable culture or massive quantities of culture filtrate. In fact we were unable in the earlier experiments to infect the rabbit even with large amounts of scarlet fever streptococci. The fact that in human scarlet fever there is so frequently a nephritic complication, presumably toxic in origin, led us to attempt to induce experimentally the kidney lesion. We assumed at the time the specific streptococcus *in vitro* would yield a soluble toxin.

Three separate isolations of the scarlet fever streptococcus were employed in our present study upon the nature of the toxic principle. Two cultures, one designated "Harrison", the other "Tyler", were supplied us by Dr. Dick of Chicago, while the third culture was one of our own which had been recovered here from the blood of a case of scarlet fever.

EXPERIMENTAL.

Separate series of rabbits were injected subcutaneously, intradermally and intravenously with varying quantities of the filtrate

of the scarlet fever streptococcus. The filtrates were obtained from nutrient broth-grown cultures and from saline suspensions of growths upon blood agar slants. The cultures were grown for 48 hours at 37° C. before they were filtered through N or V Berkfeld filters. The leucocytes and temperatures of the inoculated rabbits were noted daily for reactions, and observations were made of the areas injected intradermally. As far as we could determine no animal of this series reacted in any manner. Subsequently the animals were sacrificed and a microscopic study of the various tissues revealed nothing abnormal.

A second lot of experiments were then undertaken to determine if the toxic principle of the *streptococcus scarlatinae* did not exist in the bacterial cell. For this purpose full grown rabbits were first highly immunized by subcutaneous injections of repeated doses of 48 hour grown scarlet fever streptococci. Living cultures of the specific organism were introduced into the peritoneal cavity of these immune animals for the purpose of obtaining *in vivo* lysis. As much as 50 mils of culture were introduced intraperitoneally, which contained the saline washings of 18 to 20 cultures from blood agar slants. Three hours after the injection of culture into the peritoneum the animals were sacrificed and the peritoneal fluid collected and filtered. The filtrate was then tested for toxicity upon normal rabbits. Microscopic examination of the bacteriolysate before its filtration showed no cocci or microorganisms of any kind, and cultures prepared with 1 mil quantities of the peritoneal fluid remained sterile, which proved the complete lytic action upon the introduced microorganisms.

A third series of normal rabbits were injected with the filtrate of the peritoneal lysate. One mil quantities were given intravenously and subcutaneously, and 0.1 mil intradermally. The normal rabbits receiving the filtered lysate developed well defined symptoms and signs of toxemia within eight to twenty-four hours following the injection. The toxic effects in several of the animals proved fatal in three to five days. Many of the reacting animals showed a temperature of 107° C., high leucocytosis and later became paralyzed. There was a marked inflammatory reaction at the site of inoculation for those animals receiving the lysate intradermally. At autopsy there was revealed a swollen and congested condition of the internal organs, particularly the kidney, spleen and heart. Microscopic examination of

the tissues showed degenerative changes for the various organ parenchymes.

DISCUSSION.

Our experiments show that for the rabbit the active toxic principle of the *streptococcus scarlatinae* is intimately associated with the protoplasm of the bacterial cell, and is not given off to the artificial medium by the organism during its growth activity. Furthermore, the rabbit is highly susceptible to the lysate of the specific culture, at least for the streptococci we have employed, while entirely refractory to filtrates of actively growing cultures.

Observations upon the toxicity of certain strains of scarlet fever streptococci would indicate that as regards the rabbit, the toxic principle is more in accord with an endotoxin. Dochez's novel plan of producing toxic effects in the animal by culturing *in vivo* the streptococcus in solidified nutrient agar which had been placed in the subcutaneous tissues, does not prove the ectotoxic nature of the toxin. There are a number of bacterial species that are incapable of elaborating a soluble poison, or giving rise to a generalized infection, which under the same conditions cause toxemia in the host. Here, undoubtedly, the toxic effects are the result of the absorption of endotoxin derived from the disintegrated organisms of the *in vivo* confined culture. However, culturing the streptococcus after the method of Dochez precludes the probability that the toxic effect upon the animal used as the "incubator" originates from a systemic infection.

The animal experiments carried out by us do not support the view that the exanthem in human scarlet fever is caused by a soluble streptococcal toxin. In order to explain the "rash" it is not necessary that the infection be localized to any particular tissue or the infecting agent one that gives rise to a soluble toxin. Systemic infections are toxemias whether the causal excitant is endotoxic or ectotoxic in kind. It is questionable whether the skin-reaction to intradermal injection of specific culture filtrate, in non-immune human scarlet cases is produced by a streptococcal ectotoxin. There is no experimental evidence in the rabbit at least, to show that the scarlet fever streptococcus even under natural conditions produces what we are pleased to call a soluble toxin. Certainly the active principle is not demonstrable in the filtrate of cultures grown in nutrient broth for periods of ten days to two weeks. However, the endotoxin property of the specific

streptococcus does not preclude its accounting for the exanthem in the human case of the disease, or the "skin-reaction" in the non-immune. Even if scarlet fever is a localized infection caused by a specific streptococcus, the organisms are constantly dying, and consequently an endotoxin is liberated which eventually must reach the cutaneous tissues.

In human scarlet fever the nephritis is constant and often the outstanding feature of the disease. The glomerular lesion in the kidney is almost pathognomonic of human scarlet fever; so much so that we are inclined to regard the toxic excitant as one having a special predilection for the kidney. We have been successful in the production of a glomerular nephritis in the rabbit with *streptococcus scarlatinae* lysate.

3202

Studies upon the virus of measles

R. J. HIBBARD and C. W. DUVAL.

[*From the Department of Pathology of the Tulane University School of Medicine, New Orleans, La.*]

It is generally accepted that the symptom-complex of measles, including the exanthem and the enanthem, may be experimentally induced in the monkey, rabbit and guinea pig with either the filtrate (Berkfeld N.) of blood or naso-pharyngeal secretion from cases of human measles. The transmission experiments of Hektoen,[1] Goldberger and Anderson,[2] Blake and Trask,[3] Duval and D'Aunoy,[4] and others, have established that the causal excitant of measles is transmissible from man to lower animal, is filterable and exists in the circulating blood during the febrile stage of the disease. Although the virus of measles can be propagated in certain of the lower animals, its cultivation *in vitro* has not been definitely established. Of the various cultures reported

[1] Hektoen, L., *J. Infec. Dis.*, 1905, ii 238.

[2] Goldberger, J., and Anderson, J. F., *J. Am. Med. Assn.*, 1911, lvii, 971.

[3] Blake, F. G., and Trask, J. D., *J. Exp. Med.*, 1921, xxxiii, 385.

[4] Duval, C. W., and D'Aunoy, R., *J. Exp. Med.*, 1922, xxxv, 257; xxxvi, 231.

those of Tunnicliffe,[5] and Caronia[6] are entitled to special consideration.

With certain facts before us as a working basis, attempts were made to learn more about the nature of the measles virus; our first objective being its cultivation outside of the living host. For this purpose a variety of special as well as ordinary culture media was employed. The different media were inoculated with the filtered and unfiltered virus-blood from human measles and from the experimentally infected rabbit. Duplicate cultures were maintained under aerobic and anaerobic conditions, and incubated at a temperature of 37° C. for periods of ten days. At such times the cultures were carefully examined microscopically for growth. One mil quantities of cultures that were suggestive of growth were subplanted, and at the same time a like quantity was injected intravenously into normal rabbits. These animals were inoculated in order to determine whether the *in vitro* virus was infectious. The leucocytes and daily temperatures of these rabbits were carefully noted for reactions over a period of fourteen days.

Rabbits injected intravenously with one mil quantities of semi-solid plasma hydrocele Ringer cultures of the second, third and fourth generations reacted in a characteristic manner, *i. e.*, marked drop in the circulating leucocytes and elevation in the temperature following an incubation period of eight to twelve days. The reaction in the rabbits was identical with that induced by the injection of five mils of freshly drawn human measle blood. Here it is significant that rabbits receiving five mils of human virus blood which had been kept for two weeks at ice-box temperature failed to react. These experiments not only prove that the measles virus retains its power to infect after a relayed sojourn in hydrocele or semi-solid plasma media for a period of forty days at 37° C., but suggests that it multiplies under certain *in vitro* conditions. Since a fourth subtransplantation represents a great dilution of the original virus planted, we believe that the reaction in the animals was the result of virus numbers rather than individual virus virulence. Furthermore when the blood of rabbits which reacted to culture inoculations was injected into other rabbits these reacted similarly.

While no macroscopic growth was visible for the initial cul-

[5] Tunnicliffe, R., *J. Am. Med. Assn.*, 1917, lxviii, 1028.

[6] Caronia, *Pedicatia*, 1923, xxxi, 801.

tures or for the subsequent transplants, on careful microscopic examination there was noted in culture tubes that contained semi-solid plasma or hydrocele fluid, an occasional small Gram positive coccus. The microorganism occurred single, in pairs and short chains. Subsequently a good growth of this coccus was obtained upon ordinary blood agar slants, and under aerobic conditions at 37° C. incubation. A comparative cultural study of the various isolations revealed their complete correspondence with one another and the coccus described by Tunnicliff. In this connection it should be mentioned that in a previous study upon the transmission of measles, one of us (Duval) had noted a small Gram positive coccus in certain of the tubes of Noguchi's semi-solid medium which had been inoculated with unfiltered human measles blood. At that time the coccus was considered a contaminator and no further attention was paid to it, mainly because it occurred in cultures that had been inoculated with unfiltered measles blood and the organism appeared too large to pass through filters.

It is to be said of this coccus isolated by us from human measles blood and also of the Tunnicliff coccus that young rabbits react characteristically to intravenous injections. Cultures of the coccus were recovered from the blood of the reacting rabbits ten days after the inoculation. While no exanthem or enanthem was noted in these reacting animals, a marked concomitant leucopenia and pyrexia regularly occurred after an incubation period of seven to eight days. In regard to filterability, Tunnicliffe claims that her culture is filterable; however, she does not say under what cultural conditions; neither the coccus isolated by us from measles blood nor the coccus of Tunnicliffe when cultured aerobically will pass through the Berkfeld N or V filters. The culture filtrates fail to give rise to a reaction in the rabbit or growth upon any nutrient medium. This is significant in that it favors a causal rôle for the coccus to the exclusion of a filterable microorganism growing in association with the coccus. However, both organisms are filterable from cultures that are grown anaerobically. Under this and other abnormal growth conditions the coccus is extremely small compared to its size in cultures of favorable environment.

SUMMARY.

The causal excitant of measles exists in the circulating blood during the febrile stage of the disease. The Berkfeld filtered virus

blood induces in the monkey, rabbit and guinea pig the symptom complex of human measles.

A living agent capable of causing in the experimental animal a concomitant leucopenia and pyrexia may be propagated and subcultured *in vitro* through several generations upon hydrocele or semi-solid plasma Ringer medium. In the cultures prepared from the unfiltered human measles blood of reacting animals there frequently occurs a small gram positive coccus, which in subplants to blood agar medium grows readily and under aerobic conditions. Cultures of this coccus are likewise capable of inducing in the rabbit a leucopenia and pyrexia following an incubation period of seven to eight days. The coccus culturally and tinctorially corresponds to the culture reported by Tunnicliff. Since the coccus was cultured from the unfiltered measles blood, we prefer to reserve opinion at this writing regarding its significance in measles.

3203

The heart of the racing greyhound. Hypertrophy of the heart.

GEORGE R. HERRMANN.

[*From the Department of Medicine of the Tulane University School of Medicine, New Orleans, La.*]

The effect of strenuous competitive athletics on the heart, that is, the question of true physiological work hypertrophy, is a matter of considerable interest. A study, therefore, of the heart of the greyhound was undertaken. The hearts and body weights of ten of these thoroughbred dogs have been studied and compared with the established normal heart-weight-body-weight ratio of .00798 or 7.98 grams of heart per kilogram of body weight, for ordinary mongrel dogs.[1] The largest heart was found in the oldest and most successful racer, "Victorious Red," aged three years, with a ratio of 17.3. Five greyhounds that had had their schooling, whose age was about two years and who had raced not more than three times, ranked according to ratio as follows:

[1] Herrmann, George R., *Am. Heart J.*, 1925, i, 213.

16.2, 15.1, 14.5, 13.9, 13.6. Two dogs were full grown, aged eighteen months each, but had not been schooled and had never raced. The one of the better pedigree showed a ration of 13.4, while the other averaged 11.9. Two pups about one-third grown, aged six months each, though leading a caged up, sedentary existence, showed ratios of 11.5 and 11.3, which are above the maximum value of 9.98 found in a series of two hundred ordinary mongrel dogs.[1] The average for the greyhound series is 13.4, which is considerably above that of the stag, 11.5, which has up to this time topped the heart weight-body tables for mammals. Roentgenograms and electrocardiograms of Derby winners in the greyhound races confirmed the anatomical findings. The study suggests that the greyhound, by virtue of generations of strenuous exercise of coursing, or as a result of selection, is endowed with a proportionately large heart at birth and that this heart responds to schooling and training by hypertrophying to an unusual degree.

ERRATA.

P. 468 should read Thomas and Dutcher.
P. 471 should read Thomas and Dutcher.
P. 471, summary No. 1, should read Thomas and Dutcher.
P. 471, summary No. 5, should read Thomas and Dutcher.
The table on p. 469, fourth column, should read Thomas and Dutcher.
P. 469, heading of next to last column, should read Schaeffer, the name Quisumbing should be omitted. The heading of the last column should read Quisumbing and Thomas.

Secretary's Report, April 1, 1925, to March 31, 1926.

I. CONSTITUTION.

The Constitution was revised to make it conform more closely to the growth and national character of the Society. It was approved by the members and a By-Laws Committee appointed to prepare the by-laws.

The outstanding changes are:

1. Establishment of Associate Members.

2. Council to consist of President, Vice-President, Secretary-Treasurer, Past Presidents, and six (instead of two), elected Councillors, two retiring each year.

3. Trustees were established to be responsible for Endowment Fund.

II. OFFICERS.

A. Council.

The Council has met each month, with an average attendance of ten, including Past Presidents and Standing Committees.

The newly elected officers are:

President: James W. Jobling.

Vice-Pres.: Stanley R. Benedict.

Sec.-Treas.: A. J. Goldforb.

Six elected Councillors: Ward J. MacNeal (1927), David Marine (1927), Peyton Rous (1928), W. W. Palmer (1928), Oswald F. Avery (1929), Robert Chambers (1929).

Past Presidents: E. B. Wilson, Simon Flexner, F. S. Lee, T. H. Morgan, James Ewing, Graham Lusk, Wm. J. Gies, Gary Calkins, George B. Wallace, Holmes C. Jackson.

Chairmen of Branches:

Illinois: C. M. Child, University of Chicago.
Iowa: Victor C. Myers, State University of Iowa.
Massachusetts: Edwin J. Cohn, Harvard Medical School.
Minnesota: F. H. Scott, University of Minnesota.
Missouri: Leo Loeb, Washington University Medical School.
Pacific Coast: William Ophüls, Stanford University Medical School.
Peking: J. F. Kessel, Peking Union Medical College.
Southern: C. W. Duval, Tulane University.
Western New York: Edwin J. Bloor, University of Rochester.

B. Standing Committees.

Trustees: Gies, William J., Goldforb, A. J., Park, William H.

Membership Committee: Doisy, Edward A., Greenwald, Isidor, Wallace, G. B.

Editorial Committee: Goldforb, A. J., MacNider, W. deB., Pappenheimer, A. M., Richards, A. N., Sherman, H. C.

C. Elected Committees.

Nominating Committee: Eddy, W. H., Lusk, Gr., Marine, D., Greenwald, I., Dochez, A. R., Carlson, A. J., Myers, V. C., Wilson, D. W., Auer, J.

By-Laws Committee: Gies, W. J., Goldforb, A. J., Jackson, Holmes C., Jobling, J. W., Sherman, H. C., Wallace, G. B.

D. SECRETARIES OF BRANCHES.

Illinois: W. F. Petersen, University of Illinois Medical School.
Iowa: O. H. Gaebler, State University of Iowa.
Massachusetts: Percy G. Stiles, Harvard Medical School.
Minnesota: F. H. Scott, University of Minnesota.
Missouri: John Auer, St. Louis University School of Medicine.
Pacific Coast: T. D. Beckwith, University of California.
Peking: R. H. P. Sia, Peking Union Medical College.
Southern: J. H. Musser, Tulane University.
Western New York: Warren M. Sperry, University of Rochester Medical School

E. PAST OFFICERS, 1903-26.

	Pres.	*Vice-Pres.*	*Treas.*	*Sec.*	*Additional Members of Council.**
1903-04	Melzer	Park	Calkins	Gies	
1904-05	Melzer	Ewing	Calkins	Gies	
1905-06	Wilson	Dunham	Calkins	Gies	
1906-07	Flexner	Dunham	Calkins	Gies	
1907-08	Flexner	Morgan	Calkins	Gies	
1908-09	Lee	Morgan	Lusk	Gies	
1909-10	Lee	Gies	Lusk	Opie	
1910-11	Morgan	Gies	Lusk	Opie	
1911-12	Morgan	Levene	Lusk	Wallace	
1912-13	Ewing	Levene	Norris	Wallace	
1913-14	Ewing	Field	Norris	Jackson	
1914-15	Lusk	Gies	Murlin	Jackson	Gies, Auer
			Sec.-Treas.		
1915-16	Lusk	Calkins	Jackson		Auer, DuBois
1916-17	J. Loeb	Gies	Jackson		DuBois, Wallace
1917-18	Gies	Auer	Jackson		Wallace, Sherman
1918-19	Gies	Auer	Jackson		Sherman, Jobling
1919-20	Calkins	Wallace	Jackson		Jobling, Hess
1920-21	Calkins	Wallace	Jackson		Hess, Myers
1921-22	Wallace	Jobling	Jackson		Myers, DuBois
1922-23	Wallace	Jobling	Myers		DuBois, Benedict
1923-24	Jackson	Jobling	Goldforb		Benedict, Rous
1924-25	Jackson	Jobling	Goldforb		Benedict, Rous
1925-26	Jobling	Benedict	Goldforb		Avery, Chambers, MacNeal, Marine, Palmer, Rous.

III. MEMBERSHIP POLICY.

The Council has interpreted the clauses in the Constitution regarding Membership to mean that:

(1) The candidate must have published researches by the experimental method.

(2) The candidate shall have published in the fields of biology or medicine, not physics, engineering, analytic chemistry.

(3) Research shall be independent, or evidence shall be submitted of ability to do independent research. This applies to applicants whose titles show coauthorship only.

(4) The applicant shall have published *in extenso.*

(5) The applicant shall have done "meritorious" work.

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*Past Presidents are also members of the Council.

Persons who do not qualify by these regulations, may be elected Associates of the Branch by Branch Officers, and upon payment of $4.00 will receive the PROCEEDINGS for the year.

A Sub-Committee of the Council, on Membership, is to examine into the qualifications of applicants.

Applications from Branches are to be approved by Branch officers.

The Membership Committee is to report its findings to the Council for action.

IV. MEMBERSHIP AND SUBSCRIPTIONS.

No. of Members March 31, 1925	692		
No. of Members elected, March 31, 1925, to March 31, 1926	87		
Total membership, March 31, 1926			779
Honorary Members, March 31, 1925	3		
Honorary Members, March 31, 1926			3
No. of Subscriptions, March 31, 1925	134		
New Subscriptions, to March 31, 1926	45		
Total Subscriptions, March 31, 1926			179
Free Subscriptions, March 31, 1925	47		
Free Subscriptions, to March 31, 1926	1		
Free Subscriptions, removed, to March 31, 1926		6	
Total Free Subscriptions, March 31, 1926			42
Exchanges, March 31, 1925	9		
Exchanges withdrawn, to March 31, 1926		3	
Total exchanges, March 31, 1926			6
Total No. PROCEEDINGS distributed to March 31, 1925			885
Total No. Proceedings distributed to March 31, 1926			1009
Resignations, year ending March 31, 1926	5		
Deaths, year ending March 31, 1926	2		
Dropped for arrears, March 31, 1926	9		
Total dropped from membership list	16		

V. BRANCHES.

Illinois Branch.

Number of Members—44.

Meetings.

University of Chicago, Chicago, Ill., April 27, 1926.

Iowa Branch.

Number of Members—13.

Meetings.

State University of Iowa, November 4, 1925. Page 221.
State University of Iowa, February 3, 1926. Page 474.
State University of Iowa, April 28, 1926. Page 821.

Massachusetts Branch.

Number of Members—63.

Meetings.

Harvard Medical School, Boston, Mass., April 13, 1926. Page 694.
Massachusetts General Hospital, May 11, 1926. Page 694.

Minnesota Branch.

Number of Members—39.

Meetings.

University of Minnesota, November 4, 1925. Page 230.
University of Minnesota, December 2, 1925. Page 291.
University of Minnesota, February 3, 1926. Page 484.
University of Minnesota, April 8, 1926. Page 677.
University of Minnesota, May 5, 1926. Page 794.

Missouri Branch.

Number of Members—33.

Meetings.

Washington University School of Medicine, May 20, 1925. Page 57.
St. Louis University School of Medicine, October 28, 1925. Page 134.
Washington University School of Medicine, January 20, 1926. Page 367.
St. Louis University School of Medicine, March 3, 1926. Page 585.
Washington University School of Medicine, April 21, 1926. Page 740.
St. Louis University School of Medicine, May 26, 1926. (Vol. xxiv, No. 1.)

New York.

Number of Members—344.

Meetings.

Post-Graduate Hospital and Medical School, October 21, 1925. Page 1.
Rockefeller Institute for Medical Research, November 18, 1925. Page 81.
College of Physicians and Surgeons, Columbia, December 16, 1925. Page 167.
Cornell University Medical College, January 20, 1926. Page 251.
Presbyterian Hospital, February 17, 1926. Page 313.
University and Bellevue Medical College, March 17, 1926. Page 407.
College of the City of New York, April 21, 1926. Page 517.
Rockefeller Institute, Princeton, N. J., May 22, 1926. Page 621.

Pacific Coast Branch.

Number of Members—52.

Meetings.

University of California, October 24, 1925. Page 140.
Stanford Univ. Medical School, San Francisco, December 9, 1925. Page 297.
University of California Hospital, San Francisco, Feb. 17, 1926. Page 505.
Stanford University, Palo Alto, April 17, 1926. Page 724.
Mills College, Oakland, June 17, 1926. (Vol. xxiv, No. 1.)

Peking Branch.

Number of Members—22.

Meetings.

Peking Union Medical College, October 8, 1925. Page 242.
Peking Union Medical College, November 27, 1925. Page 388.
Peking Union Medical College, January 28, 1926. Page 606.
Peking Union Medical College, March 30, 1926. Page 668.

Southern Branch.

Number of Members—17.

Meetings.

Tulane University, October 22, 1925. Page 158.
Tulane University, February 10, 1926. Page 481.
Tulane University, May 12, 1926. Page 839.

Western New York Branch.

Number of Members—52.

Meetings.

Cornell University, October 17, 1925. Page 119.
Clifton Springs Sanitarium, Clifton Springs, N. Y., December 12, 1925. Page 282.
University of Buffalo, Buffalo, N. Y., February 13, 1926. Page 457.
University of Rochester, Rochester, N. Y., April 17, 1926. Page 714.
New York State Experiment Station, Geneva, N. Y., May 22, 1926. (Vol. xxiv, No. 1.)

Five Branches were established during the academic year, 1925-26:
Illinois Branch, Chicago, Ill.
Iowa Branch, Iowa City, Iowa.
Massachusetts Branch, Boston, Mass.
Missouri Branch, St. Louis, Mo.
Southern Branch, New Orleans, La.

Branch Secretaries' Reports.

In order to help national officers keep in close touch with Branches, the Secretary reports each meeting on the following form:

Branch Name:
Place of Meeting (Name of institution): Date:
Papers Read:
Authors: Titles:
Members present:
Members nominated:
Associates elected:
Business:

(Signed) Branch Secretary.

VI. POLICY OF THE PROCEEDINGS.

1. The PROCEEDINGS is the medium for publication of brief, preliminary or completed papers.

2. Definitive data, charts, short tables are desired rather than a general statement or general summary.

3. Highest quality, widest range of subjects are desired.

4. The Board of Editors shall approve papers before publication.

5. Manuscripts should not exceed two pages (14 inches allowed without cost to author). Papers up to five pages will be permitted. Excess space is charged at cost.

6. It is hoped that members will exercise increasing care in making their Manuscripts intelligible to other members who may be considered "laymen" in respect to their specialty.

7. Papers should conform to "Preparation of Manuscripts" prepared by the Editorial Board, printed in Volume XXIII, No. 5.

VII. TYPE OF PAPER IN PRESENT VOLUME.

To ascertain how many of the current year's papers are completed reports, and have not been or will not be published *in extenso* elsewhere, the following questionnaire was sent to each author contributing to Volume XXIII. The replies are given below:

			Per cent
1.	Is your latest paper in the PROCEEDINGS a "complete" report of research?	Yes	42
		No	58
2.	Is it a preliminary report?	Yes	69
		No	29
3.	Do you intend to publish this work elsewhere in practically its present form?	Yes	10
		No	90
4.	*In extenso?* Where?	Yes	79
		No	10
5.	Have you submitted this paper for publication elsewhere?	Yes	6
		No	88

Over 97 per cent of the questionnaires were returned.

42 per cent of the papers published are short complete papers.

The following is the list of publications to which papers will be or have been sent for publication *in extenso:*
American Journal of Anatomy.

American Journal of Clinical Research.
American Journal of Dietics.
American Journal of Diseases of Children.
American Journal of Hygiene.
American Journal of Medical Sciences.
American Journal of Pathology.
American Journal of Physiology.
American Medical Journal.
American Philos. Soc.
American Review of Tuberculosis.
American Syphylis Journal.
Anatomical Record.
Annals of Eugenics.
Annals Trop. Med. and Parasitol.
Annals of Surgery.
Archives of Internal Medicine.
Archives of Pathology.
Biologia Generalis.
Biological Bulletin.
Genetics.
Heart.
Johns Hopkins Bull.
Journal of American Chemical Society.
Journal of American Dental Association.
Journal of American Medical Association.
Journal of Bacteriology.
Journal of Biological Chemistry.
Journal of Clinical Investigation.
Journal of Comparative Neurology.
Journal of Comparative Psychology.
Journal of Experimental Biology.
Journal of Experimental Medicine.
Journal of Experimental Zoology.
Journal of General Physiology.
Journal of Immunology.
Journal of Infectious Diseases.
Journal of Lab. and Clinical Medicine.
Journal of Pharmacology.
Journal of Pharmacology and Experimental Therapy.
Journal of Stain Technology.
New Orleans Med. and Surgical Journal.
Roux's Archives.
Soil Science.
University of California Publications.

VIII. MISCELLANEOUS.

A. Editorial Board and Policy.

1. The Board of Editors have given much time and thought to examining manuscripts, formulating the policy, and making the PROCEEDINGS more useful to members. The Board consists of the following members:

Wm. deB. MacNider, University of North Carolina (Pharmacology).

A. N. Richards, University of Pennsylvania (Physiology).

A. M. Pappenheimer, Coll. of P. and S., Columbia, (Bact. and Immunol.)

H. C. Sherman, Columbia University (Biochem. and Food).

A. J. Goldforb, Coll. of City of New York (Biology).

2. Papers that reach the New York office by the third of the month, which need no extensive editing, should appear in the issue of that month. Others may appear in the next issue, or are returned to author because

(1) paper had previously appeared.
(2) paper too lengthy.
(3) paper not original research.
(4) paper too carelessly prepared.
(5) paper not experimental.

B. Evolution of Proceedings.

3. For information of members, the following data was collected from a survey of 32 consecutive Proceedings, non-selected, from the New York Academy of Medicine catalogue:

17 had changed from Proceedings to Transactions.
7 had changed from Proceedings to Journals.
5 had changed from Independent Proceedings to Proceedings published with the journal of the society.
1 had changed from Proceedings to Quarterly.
1 had changed from Proceedings to year book.
1 remained Proceedings.
0 had changed from Journal to Proceedings.

Type I. The Journal of the Royal Microscopical Society is an example. The Proceedings of this Journal is used for business announcements only.

Type II. English Journal of Physiology is an example. The Proceedings of the Journal give brief abstracts of papers of the annual meeting of the Society.

Type III. Proceedings of the National Academy of Sciences is an example. "Complete" papers are published as long as 17 pages. Each page is much larger than that of our PROCEEDINGS. They include cuts and tables.

Type IV. The *Comptes Rendus des Sciences* publishes preliminary papers 1½ to 3 pages in length, with cuts and tables. It is not clear whether any were "complete" papers.

Type V. Society for Experimental Biology and Medicine publishes papers ½ to 6 pages in length, with cuts and tables, "complete" and preliminary papers.

Type VI. Proceedings of Royal Society, publishes papers *in extenso.*

C. Indexing the Proceedings.

4. Arrangements have been made for indexing and abstracting the PROCEEDINGS in the Quarterly Cumulative Index of the American Medical Association.

IX. PROBLEMS.

1. How to make the PROCEEDINGS even more inclusive of experimental research in biology and medicine of the country.
2. How to prevent duplicate publication.
3. How to raise even higher the standards of publication.
4. How to lower costs to authors.
5. How to increase the Endowment Fund substantially.

Treasurer's Report, April 1, 1925, to March 31, 1926.

RECEIPTS.

Dues	$3336.02	
Excess space	927.04	
Reprints	1381.81	
Subscriptions	1286.96	
Sale of back numbers	329.45	
Cuts	310.72	
Endowment Fund Interest	308.35	
Miscellaneous income	61.85	
Total receipts		$7842.20
Cash Balance, April 1, 1925		917.94
Total cash available during year		$8760.14

DISBURSEMENTS.

Publication—		
Printing	$3353.39	
Reprints	987.87	
Cuts	193.94	
		$4535.20
Administrative expense—		
Office supplies	374.19	
Salaries	1281.80	
Postage	199.95	
Telegrams	20.12	
Filing cabinet	50.00	
Storage and insurance	93.84	
Refunds	35.50	
Purchase of Back Numbers	7.50	
Miscellaneous	25.06	
		2087.96
Total disbursements		$6623.16
Cash Balance, March 31, 1926		2136.98
Total cash available during year		$8760.14
Bills payable	$ 61.44	
Bills receivable	1051.17	

ENDOWMENT FUND.

In Railroad Co-op. Bldg and Loan Assn., April 1, 1925		$7886.66
Contributions, April 1, 1925, to March 31, 1926	$702.00	
Interest received, April 1, 1925, to March 31, 1926	393.77	
		1095.77
Total		$8982.43
Payment of part interest to General Fund		208.35
Balance of Endowment Fund, April 1, 1926		$8774.08

NUMBER OF MEMBERS WHO SUBSCRIBED TO ENDOWMENT FUND

Amount	*No. of members subscribing to April 1, 1926*	*Total*
$60.00	1	$ 60.00
40.00	1	40.00
35.00	3	105.00
30.00	5	150.00
25.00	7	175.00
20.00	11	220.00
15.00	206	3090.00
12.00	1	12.00
10.00	95	950.00
9.00	1	9.00
8.00	1	8.00
7.50	2	15.00
6.00	3	18.00
5.00	173	865.00
4.00	2	8.00
3.00	1	3.00
2.00	1	2.00
.50	1	.50
Total	515	$5749.00

Members who have not subscribed....264

Total active membership list.....779

BUDGET

April 1, 1926, March 31, 1927.

PROCEEDINGS	$3700.00
Administrative expense:	
Salaries	1575.00
Supplies	400.00
Postage	200.00
Telegrams	25.00
Storage and insurance	100.00
Purchasing of back numbers	25.00
Addressograph	150.00
Miscellaneous	25.00
Total	$6200.00

COST OF PROCEEDINGS, VOLUME XXII.

Gross Cost of Publishing Volume XXII (cuts and reprints deducted)	$2,734.62
Income from Excess Space	946.00
Net Cost of Publishing	$1,788.62
Income from Excess Space from Members	$ 595.00
From non-Members	381.00
Total income from Excess Space	$ 946.00

Number of pages, Volume XXII, 619.

Average net cost per page	$2.89
Present charge per page	3.50
Net cost per Volume (on basis of 885, total mailing list)	$2.02

Members' List (Alphabetical)

Honorary Members

Councilman, William T. ..... Harvard University
Reichert, Edward T. ..... University of Pennsylvania
Welch, William H. ..... Johns Hopkins University

Members

Abbott, Alexander C. ..... University of Pennsylvania
Abel, John J. ..... Johns Hopkins University
Adami, J. George ..... University of Liverpool, England
Addis, Thomas ..... Medical School, Stanford University
Adler, Herman M. ..... Juvenile Psychopathic Institute, Chicago
Alexander, Harry L. ..... Washington University
Allen, Bennet M. ..... University of California
Allen, Edgar ..... University of Missouri
Alsberg, Carl L. ..... Stanford University
Alvarez, Walter C. ..... Mayo Clinic
Amberg, Samuel ..... Mayo Clinic
Amoss, Harold L. ..... Johns Hopkins Hospital
Anderson, John F. ..... E. R. Squibb & Son
Anderson, Rudolph J. ..... N. Y. Agric. Exp. Station
Arnold, Lloyd ..... Loyola University
Asher, Leon ..... Berne, Switzerland
Ashman, Richard ..... Tulane University
Atchley, D. W. ..... Presbyterian Hospital, New York City
Atwell, Wayne J. ..... University of Buffalo, N. Y.
Aub, Joseph C. ..... Mass. General Hospital
Auer, John ..... St. Louis University
Austin, J. Harold ..... University of Pennsylvania
Avery, O. T. ..... Rockefeller Institute, N. Y. City

Baehr, George ..... Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J. ..... Memorial Hospital, N. Y. City
Bailey, C. H. ..... Columbia University
Bailey, Cameron V. ..... N. Y. Post-Graduate Medical School
Bailey, Harold C. ..... Cornell University Medical College, N. Y. City
Baitsell, George A. ..... Yale University
Baldwin, W. Manning ..... Albany Medical College
Balls, A. K. ..... University of Pennsylvania
Banta, A. M. ..... Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Banzhaf, Edwin J. ..... N. Y. Health Department
Barber, W. Howard ..... New York University Med. School
Barbour, Henry G. ..... University of Louisville
Bardeen, Charles R. ..... University of Wisconsin
Barnett, George D. ..... Stanford University
Barr, David P. ..... Washington University
Bass, Charles ..... Tulane University
Bauer, J. H. ..... Rockefeller Institute
Bauman, Louis ..... Columbia University Medical School
Baumann, E. J. ..... Montefiore Hospital, N. Y. City
Baumberger, J. Percy ..... Brussels, Belg.
Bayne-Jones, S. ..... University of Rochester
Bazett, H. C. ..... University of Pennsylvania

Becking, L. B. ..... Stanford University
Beckwith, T. D. ..... University of California
Bell, E. T. ..... University of Minnesota
Benedict, S. R. ..... Cornell University Medical College, N. Y. City
Bennitt, Rudolf ..... Tufts College
Berg, William N. ..... Berg Biological Laboratory, N. Y. City
Bergeim, Olaf ..... University of Illinois
Bergey, David H. ..... University of Pennsylvania
Bernard, Adolph ..... Lenox Hill Hospital, N. Y. City
Binger, Carl A. L. ..... Rockefeller Institute, N. Y. City
Birkhaug, Konrad E. ..... University of Rochester
Bishop, George H. ..... Clayton, Mo.
Blackfan, K. D. ..... Harvard Medical School
Blake, F. G. ..... Yale University
Blakesley, Albert F. ..... Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R. ..... Potter Metabolic Clinic, Santa Barbara, Calif.
Bloor, W. R. ..... University of Rochester
Bodansky, A. ..... Cornell University
Boeck, William C. ..... Harvard University
Bollman, Jesse L. ..... Mayo Clinic
Boothby, Walter M. ..... Kahler Hospital, Rochester, Minn.
Boots, Ralph H. ..... Rockefeller Hospital
Boring, Alice M. ..... Peking Union Medical College, China
Bostrom, Ernest F. ..... Geo. Washington University
Boyd, Julian D. ..... State University of Iowa
Boyd, Theo. E. ..... Loyola University
Brand, Irwin ..... Montefiore Hospital, New York City
Branham, Sara E. ..... University of Chicago
Brewer, Robert K. ..... Syracuse University
Briggs, A. P. ..... St. Louis University
Bronfenbrenner, J. ..... Rockefeller Institute, N. Y. City
Brooks, Clyde ..... University of Alabama
Brooks, Harlow ..... New York University
Brooks, Matilda M. ..... Hygienic Laboratory, Washington, D. C.
Brooks, S. C. ..... Hygienic Laboratory, Washington, D. C.
Brown, E. D. ..... University of Minnesota
Brown, J. Howard ..... Johns Hopkins University
Brown, Wade H. ..... Rockefeller Institute, N. Y. City
Browne, W. W. ..... College of the City of New York
Brues, Chas. T. ..... Bussey Institute, Boston
Bull, C. G. ..... Johns Hopkins University
Bunting, R. W. ..... University of Michigan
Bunting, C. H. ..... University of Wisconsin
Burnett, Theodore C. ..... University of California
Burr, Harold S. ..... Yale University
Burrows, M. T. ..... Washington University
Burton-Opitz, Russell ..... Unity Hospital
Byrne, Joseph ..... Fordham University

Carpenter, Charles M. ..... N. Y. State Veterinary College
Calkins, Gary N. ..... Columbia University
Cannon, Walter B. ..... Harvard Medical School
Carlson, A. J. ..... University of Chicago
Carter, Edward P. ..... Johns Hopkins Hospital
Cash, James R. ..... Peking Union Medical College, China
Cattell, McKeen ..... Cornell University Medical College
Caulfeild, A. H. ..... University of Toronto
Cecil, R. L. ..... Cornell University Medical College
Chace, Arthur F. ..... N. Y. Post-Graduate Medical School
Chambers, Robert ..... Cornell University Medical College

Chambers, William H.....Cornell University Medical College
Chen, K. K.....Peking Union Medical College, China
Chidester, F. E.....University of West Virginia
Child, C. M.....University of Chicago
Chittenden, R. H.....Yale University
Christian, Henry A.....Peter Bent Brigham
Churchman, John W.....Cornell University Medical School
Churchman, R. H.....Cornell University Medical College, N. Y. City
Clark, Guy W.....University of California
Clark, P. F.....University of Wisconsin
Clough, Harry .....University of Rochester
Clowes, G. H. A.....Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.....Cornell University Medical College
Cohen, Barnett.....U. S. Hygienic Laboratory, Washington, D. C.
Cohen, Martin .....N. Y. Post-Graduate Medical School
Cohn, A. E.....Rockefeller Institute, N. Y. City
Cohn, Edwin J.....Harvard Medical College
Cohn, Isadore .....New Orleans, La.
Cole, L. J.....University of Wisconsin
Cole, Rufus I.....Rockefeller Institute, N. Y. City
Cole, William H.....Clark University
Coleman, Warren .....New York University Medical College
Collens, Wm. S.....Brooklyn Jewish Hospital
Collett, Mary E.....Western Reserve University
Collier, Wm. D.....St. Louis University
Collip, J. B.....University of Alberta
Collins, Katherine R.....Spartansburg General Hospital, South Carolina
Congdon, Edgar D.....Peking Union Medical College, China
Conklin, E. G.....Princeton University
Cooke, J. V.....Washington University
Coombs, Helen C.....New York University Medical School
Cori, Carl F.....State Institute of Malignant Diseases, Buffalo, N. Y.
Corner, George W.....University of Rochester
Coulter, Calvin B.....Columbia University
Couret, M. J.....Tulane University
Cowan, George V.....Stanford University Medical School
Cowgill, George R.....Yale University
Crampton, C. Ward.....N. Y. Post-Graduate Medical School
Crile, George W.....Western Reserve University
Crohn, Burrill B.....Mt. Sinai Hospital, N. Y. City
Croll, Hilda M.....University of Illinois
Crozier, W. J.....Harvard University
Cruickshank, E. W. H.....King's College, Cambridge, Eng.
Csonka, F. A.....U. S. Bureau Chemistry, Washington, D. C.
Cullen, Glenn E.....Vanderbilt University
Cummins, Harold .....Tulane University
Cunningham, R. S.....Vanderbilt University
Curtis, Maynie R.....Columbia University

Dack, Gail M.....University of Chicago
Dakin, H. D.....Ossining, N. Y.
Danforth, Chas. H.....Stanford University
Daniels, Amy L.....University of Iowa
Danzer, Charles S.....N. Y. Homeopathic Medical College
Davenport, C. B.....Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davies, H. Whitridge.....University of Edinburgh
Davis, D. J.....University of Illinois
Davison, Wilburt C.....Johns Hopkins University
Dawson, James A.....Harvard University
De Bord, George G.....Oklahoma A. & M. College

De Eds, Floyd ..... Stanford University Medical School
De Graf, A. C. ..... N. Y. University Medical School
Denis, Willey ..... Tulane University
Derick, C. L. ..... Rockefeller Institute
Detwiler, S. R. ..... Harvard University
Deuel, Harry J. Jr. ..... Cornell University Medical College
Dickson, E. C. ..... Stanford University Medical School
Dieuaide, Francis R. ..... Peking Union Medical College, China
Doan, Charles A. ..... Rockefeller Institute
Dochez, A. R. ..... Presbyterian Hospital, N. Y. City
Doisy, Edward A. ..... St. Louis University
Dolley, David H. ..... St. Louis University
Donaldson, H. H. ..... Wistar Institute, Philadelphia
Dooley, M. S. ..... Syracuse University
Drabkin, D. L. ..... Yale University
Dragstedt, Lester R. ..... Northwestern University
Draper, George W. ..... Columbia University
Draper, John W. ..... New York City
Dresbach, M. ..... Albany Medical College
Dubin, Harry E. ..... Metz Laboratories, N. Y. City
DuBois, E. F. ..... Cornell University Medical College
Duggar, B. M. ..... Missouri Botanical Gardens
Dunn, Halberg L. ..... Baltimore, Md.
Dunn, L. C. ..... Storrs Agr. Exp. Station
Dunn, Max S. ..... University of S. California
Dutcher, R. Adams ..... Pennsylvania State College
Duval, C. W. ..... Tulane University

Eberson, Frederick C. ..... University of California Hospital
Eckles, C. H. ..... University of Minnesota
Eddy, Walter H. ..... Columbia University
Edmunds, C. W. ..... University of Michigan
Edwards, D. J. ..... Cornell University Medical College
Eggston, Andrew A.,
Manhattan Eye, Ear, Nose and Throat Hospital, N. Y. City.
Eisberg, Harry B. ..... N. Y. University and Bellevue Medical College
Eisenbray, A. B. ..... Western Reserve University
Elsberg, Charles A. ..... Mt. Sinai Hospital, N. Y. City
Elser, W. J. ..... Cornell University Medical College
Embrey, Hartley C. ..... Chattanooga, Tenn.
Emmil, V. E. ..... University of Illinois
Epstein, A. A. ..... Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda ..... University of Berlin, Germany
Erlanger, Joseph ..... Washington University
Evans, Herbert M. ..... University of California
Ewing, James ..... Cornell University Medical College
Eyster, J. A. E. ..... University of Wisconsin

Faber, Harold K. ..... Stanford University Medical School
Fahr, George ..... University of Minnesota
Falk, K. George ..... Roosevelt Hospital, N. Y. City
Falk, I. S. ..... University of Chicago
Falls, Frederick H. ..... University of Iowa
Famulener, L. W. ..... St. Luke's Hospital, N. Y. City
Faust, Ernest C. ..... Peking Union Medical College, China
Felton, L. D. ..... Harvard University
Ferry, R. M. ..... Harvard University
Field, Cyrus W. ..... New York City
Fine, M. S. ..... Battle Creek, Mich.
Fischer, Albert ..... University of Copenhagen, Denmark
Fischer, Martin H. ..... University of Cincinnati

Fish, Pierre A. ..... Cornell University
Fitch, C. P. ..... University of Minnesota
Fitz, Reginald ..... Peter Bent Brigham Hospital
Fitzgerald, J. G. ..... University of Toronto
Fleisher, Moyer S. ..... St. Louis University
Fleischner, E. C. ..... University of California
Flexner, Simon ..... Rockefeller Institute, N. Y. City
Florence, Laura ..... N. Y. Homeopathic Medical School
Flournoy, Thomas ..... House of Mercy Hospital, Pittsfield, Mass.
Forbes, Alexander ..... Harvard University
Foster, Goodwin L. ..... University of California
Foster, Nellis B. ..... New York Hospital, N. Y. City
Frankel, Florence Hulton ..... Montefiore Hospital, N. Y. City
Freedman, Louis ..... Metz Laboratory
Friedman, G. A. ..... Columbia University
Fridericia, L. S. ..... University of Copenhagen, Denmark
Funk, Casimir ..... State Institute of Hygiene, Poland

Gaebler, O. H. ..... University of Iowa
Gager, C. Stuart ..... Brooklyn Botanic Garden, Brooklyn, N. Y.
Gamble, James L. ..... Harvard University
Garbat, Abraham L. ..... Lenox Hill Hospital, N. Y. City
Garrey, Walter E. ..... Vanderbilt University
Gasser, Herbert S. ..... Washington University
Gates, Frederick L. ..... Rockefeller Institute
Gay, F. P. ..... Columbia University
Gesell, Robert A. ..... University of Michigan
Gettler, A. O. ..... University and Bellevue Medical College
Geyelin, Henry R. ..... Columbia University
Gibson, R. B. ..... University of Iowa
Gies, William J. ..... Columbia University
Githens, T. S. ..... Mulford Company, Philadelphia, Pa.
Givens, Maurice H. ..... Northwestern Yeast Co., Chicago
Glasser, Otto ..... Amherst College
Goldberg, S. A. ..... Cornell University
Goldforb, A. J. ..... College of the City of New York
Goldschmidt, Samuel ..... University of Pennsylvania
Gortner, R. A. ..... University of Minnesota
Gould, Harley N. ..... Tulane University
Graham, Evarts A. ..... Washington University
Graves, William W. ..... St. Louis University
Green, Robert G. ..... University of Minnesota
Greenberg, David N. ..... University of California
Greenwald, Isidor ..... Roosevelt Hospital, N. Y. City
Gregory, Louise H. ..... Barnard College, Columbia University
Griffith, Fred R., Jr. ..... University of Buffalo
Griffith, Wendel ..... St. Louis University
Gross, Erwin G. ..... Yale University
Gross, Louis ..... Brownsville Hospital, N. Y. City
Gruber, Charles M. ..... Washington University
Guenther, A. E. ..... University of Nebraska
Guthrie, C. C. ..... University of Pittsburgh
Guttmacher, A. F. ..... Johns Hopkins Hospital
Guy, Ruth A. ..... Peking Union Medical College, China

Hadley, Philip ..... University of Michigan
Hagan, William Arthur ..... Cornell University
Hale, Worth ..... Harvard Medical School
Hall, Ivan C. ..... University of Colorado
Halsey, John ..... New Orleans, La.

Halsey, Robert H.....N. Y. Post-Graduate Medical School
Hamburger, W.....Rush Medical College
Hamilton Benton.....Children's Hospital, Boston
Hammett, F. S.....Wistar Institute
Hanzlik, P. J.....Leland Stanford University
Hardesty, Irving.....Tulane University
Harris, Isaac F.....Tuckahoe, N. Y.
Harris, J. Arthur.....University of Minnesota
Harris, William H.....Tulane University
Harrison, R. G.....Yale University
Harrop, George A. Jr.....Johns Hopkins University
Harrow, Benjamin.....Columbia University
Hartman, Carl G.....Johns Hopkins University
Hartman, F. A.....University of Buffalo
Hartwell, John A.....Cornell University Medical College
Harvey, E. Newton.....Princeton University
Harvey, Samuel C.....Yale University
Hastings, A. Baird.....Rockefeller Institute, N. Y. City
Hatai, Shinkishi.....Tohoku Imperial University, Japan
Hatcher, R. A.....Cornell University Medical College
Hawk, P. B.....N. Y. City
Hayden, Charles E.....Cornell University
Haythorn, Samuel R.....University of Pittsburgh
Heft, Hattie L.....Columbia University
Heidelberger, Michael.....Rockefeller Institute
Helmholz, Henry R.....Mayo Clinic
Hench, Philip S.....Mayo Clinic
Henderson, Lawrence J.....Harvard University
Hendrix, B. M.....University of Texas
Henrici, Arthur T.....University of Minnesota
Herrman, G. R.....Tulane University
Hess, Alfred F.....N. Y. University and Bellevue Medical School
Heymans, J. F.....U. de Gand, Belgium
Hewlett, A. W.....Lane Hospital, San Francisco
Hill, Eban C.....Johns Hopkins University
Hines, H. M.....State University of Iowa
Hirschfelder, Arthur.....University of Minnesota
Hoffman, George L.....Allegheny County Hospital, Pittsburgh, Pa.
Hoffman, W. F.....Cloquet, Minn.
Holm, George E.....Department of Agriculture, Washington, D. C.
Holman, W. L.....University of Toronto
Holmes, S. J.....University of California
Holt, L. Emmett, Jr.....Johns Hopkins University
Hooker, Davenport.....University of Pittsburgh
Hooker, Sanford B.....Boston University
Hooper, Charles W.....Metz Laboratories
Hopkins, J. Gardner.....Columbia University
Hoskins, R. G.....Ohio State University
Höst, H. F.....Horik, Norway
Howard, Harvey J.....Peking Union Medical College, China
Howe, Paul E.....Department of Agriculture, Washington, D. C.
Howell, William H.....Johns Hopkins University
Howell, K. M.....Michael Reese Hospital
Howland, John.....Johns Hopkins Hospital
Hubbard, Roger S.....Clifton Springs Sanitarium, N. Y.
Huber, G. Carl.....University of Michigan
Hunt, Reid.....Harvard University
Hunter, Andrew.....University of Toronto
Huntoon, F. M.....Mulford Co., Glenolden, Pa.
Hurwitz, Samuel.....University of California

Irving, Lawrence ..... Frankfort, Germany
Irwin, Marion ..... Rockefeller Institute
Ivy, Andrew C. ..... Northwestern University

Jackson, C. M. ..... University of Minnesota
Jackson, D. E. ..... University of Cincinnati
Jackson, Henry, Jr. ..... Boston City Hospital
Jackson, Holmes C. ..... N. Y. University and Bellevue Medical School
Jacobs, Walter A. ..... Rockefeller Institute, N. Y. City
Jaffe, Henry L. ..... Hospital for Joint Diseases, N. Y. City
Jaffe, Richard H. ..... University of Illinois
Jeans, Philip C. ..... University of Iowa
Jennings, H. S. ..... Johns Hopkins University
Jobling, J. W. ..... Columbia University
Johns, Foster M. ..... Tulane University
Jonas, Leon ..... University of Pennsylvania
Jones, Frederick S. ..... Rockefeller Institute, Princeton, N. J.
Jordan, Edwin ..... University of Chicago
Jordan, H. E. ..... University of Virginia
Joseph, Don R. ..... St. Louis University
Joslin, E. P. ..... Boston, Mass.

Kahn, Morris H. ..... Beth Israel Hospital, N. Y. City
Kahn, Morton C. ..... Cornell Medical College, N. Y. City
Kahn, R. L. ..... Michigan Department of Health, Lansing
Karsner, H. T. ..... Lakeside Hospital, Cleveland
Kast, Ludwig ..... N. Y. Post-Graduate Medical School
Keeton, Robert W. ..... University of Illinois
Kellogg, V. L. ..... National Research Council, Washington, D. C.
Kendall, Arthur I. ..... Washington University Medical School
Kendall, E. C. ..... Mayo Clinic, Minn.
Kessel, John F. ..... Peking Union Medical College, China
Key, John A. ..... Shriner Hospital, St. Louis, Mo.
Killian, J. A. ..... N. Y. Post-Graduate Medical School
Kingsbury, F. B. ..... Metropolitan Life Insurance Co., N. Y. City
Kinsella, Ralph A. ..... St. Louis University
Kirkbride, Mary B. ..... N. Y. State Dep't of Health, Albany, N. Y.
Kirkham, William B. ..... Springfield College, Mass.
Kleiner, I. S. ..... N. Y. Homeopathic Medical School
Kleitman, Nathaniel ..... University of Chicago
Kligler, I. J. ..... Hebrew University, Palestine
Kline, B. S. ..... Mt. Sinai Hospital, Cleveland, Ohio
Klotz, Oskar ..... University of Toronto
Knowlton, Frank P. ..... Syracuse University
Knudson, Arthur ..... Albany Medical College
Koch, Elizabeth ..... University of Illinois
Koch, Fred C. ..... University of Chicago
Koch, Mathilda L. ..... Greenwich, Conn.
Kocher, R. A. ..... San Diego, Calif.
Kofoid, Charles A. ..... University of California
Kolmer, John A. ..... University of Pennsylvania
Kopeloff, Nicholas ..... Psychiatric Institute, N. Y. City
Koppányi, Theodore ..... University of Chicago
Korns, John H. ..... Peking Union Medical College, China
Koser, Stewart ..... University of Illinois
Kramer, Benjamin ..... Brooklyn Jewish Hospital
Krumbhaar, E. B. ..... Philadelphia General Hospital, Philadelphia
Krumwiede, Charles ..... N. Y. City Dept. of Health
Kruse, Theophile K. ..... University of Pittsburgh
Kugelmass, I. Newton ..... Yale University

Kunde, Marg. ..... University of Chicago
Kuntz, Albert ..... St. Louis University
Kuttner, A. G. ..... Rockefeller Institute

Ladd, William S. ..... Presbyterian Hospital
Lamar, R. V. ..... University of Georgia
Lambert, R. A. ..... School of Tropical Medicine, San Juan
La Mer, Victor K. ..... Columbia University
Lamson, Paul D. ..... Vanderbilt University
Lancefield, D. E. ..... Columbia University
Landsteiner, Karl ..... Rockefeller Institute, N. Y. City
Langstroth, Lovell ..... University of California
Larson, J. A. ..... Psychopathic Institute, Chicago
Larson, W. P. ..... University of Minnesota
Lashley, K. S. ..... University of Minnesota
Lathrop, Carl O. ..... University of Buffalo
Laughlin, H. H. ..... Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Laurens, Henry ..... Tulane University
Leake, J. P. ..... Hygienic Laboratory, Washington, D. C.
Lee, Ferdinand C. ..... Johns Hopkins University
Lee, Frederick S. ..... Columbia University
Levene, P. A. ..... Rockefeller Institute, N. Y. City
Levene, Victor ..... Creighton University
Levin, Isaac ..... New York City
Levine, Michael ..... Montefiore Hospital, N. Y. City
Levinson, Samuel A. ..... University of Illinois
Levy, Robert L. ..... Presbyterian Hospital, N. Y. City
Lewis, Howard B. ..... University of Michigan
Lewis, Paul A. ..... Rockefeller Institute, Princeton, N. J.
Lewis, Robert C. ..... University of Colorado
Liddel, H. S. ..... Cornell University
Lieb, C. C. ..... Columbia University
Lillie, Frank R. ..... University of Chicago
Lillie, Ralph S. ..... University of Chicago
Lim, Robert K. ..... Peking Union Medical College, China
Lipman, Charles B. ..... University of California
Little, C. C. ..... University of Michigan
Liu, J. Heng ..... Peking Union Medical College, China
Loeb, Leo ..... Washington University
Loeb, Robert F. ..... Presbyterian Hospital, N. Y. City
Loevenhart, A. S. ..... University of Wisconsin
Lombard, Warren P. ..... University of Michigan
Longcope, W. T. ..... Johns Hopkins University
Lucas, William P. ..... University of California
Lucke, Baldwin ..... University of Pennsylvania
Luckhardt, A. B. ..... University of Chicago
Lund, E. J. ..... University of Minnesota
Lundsgaard, Christen ..... Medical Clinic A, Copenhagen, Denmark
Lusk, Graham ..... Cornell University Medical College
Lyle, W. G. ..... Roosevelt Hospital, N. Y. City
Lynch, Clara J. ..... Rockefeller Institute, N. Y. City
Lyon, E. P. ..... University of Minnesota

Marriott, McKim ..... Washington University
McCann, William S. ..... University of Rochester
McClendon, J. Francis ..... University of Minnesota
McClintock, John T. ..... University of Iowa
McCollum, E. V. ..... Johns Hopkins University
McCrudden, Francis H. ..... Boston, Mass.
McCutcheon, Morton ..... University of Pennsylvania

McEllroy, W. S. ..... University of Pittsburgh
McIver M. A. ..... Mass. General Hospital
McJunkin, Frank A. ..... Washington University
McKinley, E. B. ..... Columbia University

McLean, Franklin C. ..... University of Chicago
McMaster, Philip D. ..... Rockefeller Institute, N. Y. City
McMeans, J. W. ..... Pittsburgh Hospital
McQuarrie, Irvine ..... Yale University
MacDougal, D. T. ..... Desert Laboratory, Tucson, Arizona
MacDowell, E. Carlton ..... Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
MacLeod, Grace ..... Columbia University
MacLeod, J. J. R. ..... University of Toronto
MacNeal, Ward J. ..... N. Y. Post-Graduate Medical School
MacNider, William de B. ..... University of North Carolina
Macallum, A. B. ..... McGill University
Macht, David I. ..... Johns Hopkins University
Mackenzie, George M. ..... Presbyterian Hospital
Magath, T. B. ..... Mayo Clinic, Minn.
Mallory, Frank ..... Boston City Hospital
Maltaner, Frank ..... N. Y. State Department of Health, Albany
Mandel, Arthur R. ..... N. Y. University and Bellevue Medical College
Mandel, John A. ..... N. Y. University and Bellevue Medical College
Mann, Frank C. ..... Mayo Clinic
Mann, Hubert ..... United States Veterans' Hospital, N. Y. City
Manwaring, W. H. ..... Stanford University Medical School
Marine, David ..... Montefiore Hospital, N. Y. City
Marriott, McK. ..... St. Louis Children's Hospital
Marshall, E. K., Jr. ..... Johns Hopkins University
Martin, E. G. ..... Leland Stanford University
Matas, Rudolph ..... New Orleans, La.
Mattill, Henry A. ..... University of Rochester
Mavor, James A. ..... Union College
Maximow, Alexander A. ..... University of Chicago
Maynard, L. A. ..... Cornell University
Means, J. H. ..... Mass. General Hospital
Megrail, Emerson ..... Western Reserve University
Mehrtens, Henry G. ..... Stanford University Medical School
Meigs, E. B. ..... Dairy Division Exp. Station, Beltsville, Md.
Meleney, F. L. ..... Presbyterian Hospital
Meleney, Henry E. ..... Peking Union Medical College, China
Mellon, Ralph R. ..... Highland Hospital, Rochester, N. Y.
Mendel, Lafayette B. ..... Yale University
Mendel, W. L. ..... Harvard University
Menten, Maude L. ..... University of Pittsburgh
Metz, Charles W. ..... Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Meyer, Adolf ..... Johns Hopkins University
Meyer, A. L. ..... Johns Hopkins University
Meyer, A. W. ..... Stanford University Medical School
Meyer, G. M. ..... Rockefeller Institute, N. Y. City
Meyer, K. F. ..... University of California
Miles, L. M. ..... Peking Union Medical College, China
Miller, C. Philip, Jr. ..... Paris, France
Miller, G. H. ..... University of Iowa
Millet, John A. P. ..... Stockbridge, Mass.
Mitchell, O. W. H. ..... Syracuse University
Moore, A. R. ..... Rutgers College
Morgan, T. H. ..... Columbia University
Morse, Arthur ..... New Haven Hospital
Morse, Withrow ..... Jefferson Medical College

Mosenthal, H. O.....N. Y. Post-Graduate Medical School
Moss, W. L.....Harvard University
Mudd, Stuart .....University of Pennsylvania
Mudge, B. S.....University of California
Mueller, E. F.....Columbia University
Mueller, J. Howard.....Harvard University
Muller, Herman J.....University of Texas
Murlin, John R.....University of Rochester
Murphy, J. B.....Rockefeller Institute
Murray, Henry A.....N. Y. City
Murray, Thomas J.....Rutgers College
Musser, John H.....Tulane University
Myers, Chester N.....Columbia University
Myers, Victor C.....University of Iowa

Nelson, Thurlow C.....Rutgers College
Nicholas, J. S.....University of Pittsburgh
Niles, Walter L.....Cornell University Medical College
Noble, W. C., Jr.....N. Y. State Board of Health, N. Y. City
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Norris, Charles .....Chief Medical Examiner, N. Y. City
Northrup, John H.....Rockefeller Institute, N. Y. City
Norton, J. F.....University of Chicago
Novy, Frederick G.....University of Michigan
Nye, R. N.....Boston City Hospital

Oertel, Horst.....McGill University
Olitsky, Peter K.....Rockefeller Institute, N. Y. City
Oliver, Jean .....Lane Hospital, San Francisco, Calif.
Ophüls, William .....Stanford University Medical College
Opie, Eugene L.....Henry Phipps Institute, Philadelphia
Oppenheimer, B. S.....Columbia University
Ornstein, George G.....Columbia University
Osborne, Thomas B.....Agricultural Exp. Station, New Haven, Conn.
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Packard, Charles .....Columbia University
Page, Irvine H.....Cornell University Medical College
Palmer, Leroy S.....University of Minnesota
Palmer, W. W.....Presbyterian Hospital, N. Y. City
Papanicolaou, George N.....Cornell University Medical College
Pappenheimer, A. M.....Columbia University
Park, E. A.....Yale University
Park, William H.....N. Y. University and Bellevue Medical College
Parker, George H.....Harvard University
Parker, Julia T.....Columbia University
Peabody, Francis W.....Boston City Hospital
Pearce, Louise .....Rockefeller Institute
Pearl, Raymond .....Johns Hopkins University
Pease, Marshall C.....N. Y. Post-Graduate Medical School
Peirce, George J.....Stanford University Medical School
Pellini, Emil J.....N. Y. University and Bellevue Medical College
Pemberton, Ralph .....Presbyterian Hospital, Philadelphia
Penfield, Wilder G.....Columbia University
Pepper, O. H. Perry.....University of Pennsylvania
Perlzweig, William A.....Johns Hopkins Hospital
Permar, Howard H.....Mercy Hospital, Pittsburgh
Peters, John P.....Yale University

Peterson, W. F.....University of Illinois Medical College
Pettibone, C. J. V.....University of Minnesota
Pfaff, Franz .....Harvard University
Pfeiffer, J. A. F.....Johns Hopkins University
Pike, F. H.....Columbia University
Plant, O. H.....University of Iowa
Plotz, Harry .....Pasteur Institute, Paris
Pohlman, Augustus G.....St. Louis University
Porter, William T.....Harvard University
Powers, Grover F.....Yale University
Pratt, Fred H.....Boston University
Pratt, Joseph H.....Boston, Mass.
Prewitt, Proviso .....N. Y. University and Bellevue Medical College
Prince, A. L.....Hartford Hospital, Conn.

Quinby, W. C.....Peter Bent Brigham Hospital

Raiziss, George W.....Research Institute of Cutaneous Medicine, Philadelphia
Rakestraw, Norris W.....Oberlin College
Rasmussen, A. T.....University of Minnesota
Ratner, Bret .....N. Y. University and Bellevue Medical College
Ravenel, M. P.....University of Missouri
Ray, Henry M.....South Side Hospital, Pittsburgh
Read, Bernard E.....Peking Union Medical College, China
Read, J. Marion.....Stanford University Medical School
Redfield, A. C.....Harvard University
Reed, Carlos I.....Baylor University Medical College
Reiman, Stanley P.....University of Pennsylvania
Rettger, L. F.....Yale University
Reznikoff, Paul .....Cornell University
Richards, Alfred N.....University of Pennsylvania
Richards, Herbert M.....Columbia University
Richardson, Henry B.....Cornell University Medical College
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Ringer, Michael .....Montefiore Hospital, N. Y. City
Robertson, H. E.....University of Minnesota
Robertson, Oswald H.....Peking Union Medical College, China
Robertson, T. B.....University of Adelaide, South Australia
Robinson, Charles S.....Michigan Agricultural Station, Lansing
Robinson, Elliott .....Antitoxin Laboratory, Boston
Robinson, G. Canby.....Vanderbilt University
Rockwood, Elbert W.....University of Iowa
Rogers, Fred T.....Baylor University
Rogoff, J. M.....Western Reserve University
Rhodenburg, George L.....Lenox Hill Hospital
Roman, Benjamin .....Buffalo General Hospital, N. Y.
Rose, Anton R.....Prudential Life Insurance Co., Newark, N. J.
Rose, Mary Swartz.....Columbia University
Rose, William C.....University of Illinois
Rosenau, M. J.....Harvard Medical School
Rosenow, E. C.....Mayo Foundation
Ross, Victor .....Lehn and Fink, Bloomfield, N. J.
Roth, George B.....George Washington University
Rothschild, M. A.....Mt. Sinai Hospital, N. Y. City
Rous, Peyton .....Rockefeller Institute
Rowe, Allan W.....Boston University
Ryan, A. H.....Tufts Medical College

Sabin, Florence R. ..... Rockefeller Institute
Salant, William ..... University of Georgia
Salvesen, Harald A. ..... Rikshospitalet, Oslo, Norway
Sandburg, M. ..... Montefiore Hospital
Sanford, A. H. ..... Mayo Clinic
Sansum, W. D. ..... Santa Barbara Cottage Hospital
Scammon, R. E. ..... University of Minnesota
Schick, Bela ..... Mount Sinai Hospital, N. Y. City
Schlesinger, M. J. ..... Harvard University

Schloss, Oscar M. ..... Cornell University Medical College
Schlutz, F. W. ..... University of Minnesota
Schmidt, Carl F. ..... University of Pennsylvania
Schmidt, Carl L. A. ..... University of California
Schneider, Edward C. ..... Wesleyan University
Schultz, E. W. ..... Leland Stanford University
Schultz, O. T. ..... University of Chicago
Schultz, W. H. ..... University of Maryland
Schwyzer, Fritz ..... Kastanienbaum, Switzerland
Scott, E. L. ..... Columbia University
Scott, F. H. ..... University of Minnesota
Scott, G. G. ..... College of the City of New York
Scott, R. W. ..... City Hospital, Cleveland, Ohio
Senior, Harold D. ..... N. Y. University and Bellevue Medical College
Shaffer, Philip A. ..... Washington University
Shaklee, A. O. ..... St. Louis University
Shannon, W. R. ..... University of Minnesota
Sharlit, Herman ..... Roosevelt Hospital, N. Y. City
Sharp, P. E. ..... Cornell University
Sherman, H. C. ..... Columbia University
Sherman, James M. ..... Cornell University
Sherwin, Carl P. ..... Fordham University
Shevky, Eshref ..... Stanford University Medical College
Shibley, Gerald S. ..... Columbia University
Shiple, George. J., S. J. ..... Woodstock College, Md.
Shohl, Alfred T. ..... Yale University
Sia, Richard H. P. ..... Peking Union Medical College, China
Siler, J. F. ..... United States Army
Simpson, George Eric ..... University of Pennsylvania
Sittenfield, M. J. ..... Columbia University
Smith, Arthur H. ..... Yale University
Smith, Clarence ..... N. Y. City
Smith, Fred M. ..... University of Iowa
Smith, George H. ..... Yale University
Smith, Millard ..... Harvard University
Smith, Philip E. ..... University of California
Smith, Theobald ..... Rockefeller Institute, Princeton, N. J.
Smyly, H. Joselyn ..... Peking Union Medical College, China
Snyder, Franklin F. ..... University of Rochester
Sollmann, Torald ..... Western Reserve University
Soule, M. H. ..... University of Michigan
Speidel, C. C. ..... University of Virginia
Stakman, E. C. ..... University of Minnesota
Stark, Mary B. ..... N. Y. Homeopathic Medical College, N. Y. City
Starkey, R. L. ..... University of Minnesota
Stevens, Franklin A. ..... Presbyterian Hospital, N. Y. City
Stewart, G. N. ..... Western Reserve Medical School
Stiles, Percy G. ..... Harvard University
Stillman, Edgar G. ..... Presbyterian Hospital, N. Y. City
Stillman, Ralph G. ..... Cornell University

Stockard, Charles R. ..... Cornell University Medical College
Stookey, Lyman B. ..... University of Southern California
Storey, Thomas A. ..... College of the City of New York
Strong, Richard P. ..... Harvard University
Strouse, Solomon ..... Northwestern University
Sturgis, C. C. ..... Peter Bent Brigham Hospital
Sturtevant, A. H. ..... Columbia University
Suguira, Kanematsu ..... Memorial Hospital, N. Y. City
Sumner, James B. ..... Cornell University
Sundstroem, Edward S. ..... University of California
Swain, R. E. ..... Leland Stanford University
Sweet, J. Edmund ..... University of Pennsylvania
Swett, Francis H. ..... Vanderbilt University
Swift, H. F. ..... Rockefeller Institute, N. Y. City
Swingle, W. W. ..... Yale University
Symmers, Douglas ..... N. Y. University and Bellevue Medical College

Talbot, Fritz B. ..... Harvard University
Tashiro, Shiro ..... University of Cincinnati
Tatum, A. L. ..... University of Chicago
Taylor, Charles V. ..... Leland Stanford University
Ten-Broeck, Carl ..... Peking Union Medical College, China
Terry, B. T. ..... Toledo Hospital
Thatcher, Robert W. ..... N. Y. Agr. Exp. Station, Geneva, N. Y.
Thomas, Arthur W. ..... Columbia University
Thomas, J. E. ..... St. Louis University
Thomas, Karl ..... Physiologish-Chemisches Institut, Leipzig, Germany
Thomas, Walter S. ..... Clifton Springs Sanitarium, N. Y.
Thro, William C. ..... Cornell University Medical College
Tisdall, Frederick F. ..... University of Toronto
Tolstoi, Edward ..... Yale University
Torrey, Harry B. ..... Cornell Univ. Medical School
Torrey, J. C. ..... Cornell University Medical College
Towne, Edward B. ..... Lane Hospital, San Francisco
Tsen, Edgar T. H. ..... Epidemic Prevention Bureau, Peking, China
Tso, Ernest ..... Yale University
Tyzzer, E. E. ..... Harvard University

Uhlenhuth, Eduard ..... University of Maryland
Underhill, Frank P. ..... Yale University

Van Slyke, Donald D. ..... Rockefeller Institute
Vaughan, T. Wayland ..... Scripps Institution, California
Vogel, Karl M. ..... Columbia University
Von Meysenburg, Ludo ..... Tulane University

Wadsworth, Augustus B. ..... N. Y. State Department of Health
Waksman, S. A. ..... N. J. State Agr. Experiment Station
Walker, E. L. ..... University of California
Wallace, George B. ..... N. Y. University and Bellevue Medical College
Wang, Chi Che ..... University of Chicago
Warden, Carl C. ..... St. Joseph's Hospital, Ann Arbor, Mich.
Warthin, Alfred S. ..... University of Michigan
Wasteneys, H. ..... University of Toronto
Watanabe, C. K. ..... Watanabe Hospital, Tokyo, Japan
Wearn, J. T. ..... Boston City Hospital
Webster, Leslie T. ..... Rockefeller Institute
Weiskotten, Herman G. ..... Syracuse University
Weiss, Charles ..... Columbia University
Weiss, Harry ..... Columbia University

Weiss, Soma ..... Boston City Hospital
Welker, W. H. ..... University of Illinois Medical College
Weller, Carl V. ..... University of Michigan
West, Randolph ..... Presbyterian Hospital, N. Y. City
Weymouth, Frank W. ..... Leland Stanford University
Wheeler, Wm. M. ..... Bussey Institute
Whipple, George H. ..... University of Rochester
White, G. Benjamin ..... Antitoxin and Vaccine Laboratory, Boston
White, H. L. ..... Washington University
White, Orland E. ..... Brooklyn Botanic Garden, Brooklyn, N. Y.
Wible, Charles L. ..... N. Y. University and Bellevue Medical College
Wiggers, Carl J. ..... Western Reserve University
Willaman, J. J. ..... University of Minnesota
Williams, Anna W. ..... Department of Health, N. Y. City
Williams, Horatio B. ..... Columbia University
Williams, H. U. ..... University of Buffalo
Williams, J. R. ..... Highland Hospital, Rochester, N. Y.
Williams, Robert R. ..... Western Electric Company
Wilson, D. Wright ..... University of Pennsylvania
Wilson, Edmund B. ..... Columbia University
Wilson, E. Bidwell ..... Harvard University
Williamson, C. S. ..... University of Illinois Medical College
Winslow, C. E. A. ..... Yale University
Winternitz, Milton C. ..... Yale University
Wislocki, George B. ..... Johns Hopkins University
Wolbach, S. B. ..... Harvard University
Wolf, C. G. L. ..... Addenbrooke's Hospital, Cambridge, England
Wollstein, Martha ..... Babies' Hospital, N. Y. City
Wood, Francis C. ..... Columbia University
Woodruff, L. L. ..... Yale University
Wu, Hsien ..... Peking Union Medical College, China

Yatsu, Naohide ..... Zoological Institute, Tokyo, Japan
Yerkes, Robert M. ..... Yale University
Youland, William E., Jr. ..... N. Y. Homeopathic Medical College
Young, C. C. ..... Department of Health, Mich.
Young, Charles W. ..... Peking Union Medical College, China

Zingher, Abraham ..... Department of Health, N. Y. City
Zinsser, Hans ..... Harvard University
Zucker, Theodore ..... Columbia University

Total number of members at the close of the academic year, 1925-26: 779.

Members' List (By Branches).

Honorary Members.

William T. Councilman, Harvard University.
Edward T. Reichert, University of Pennsylvania.
William H. Welch, Johns Hopkins University.

New York Society.

Carnegie Institute of Washington—Banta, A. M., Davenport, C. B., Laughlin, H. H., MacDowell, E. C., Metz, C. W., Riddle, O.

College of the City of New York—Browne, W. W,. Goldforb, A. J., Scott, G. G., Storey, T. A.

Columbia University—Calkins, G. N., Coulter, C. B., Eddy, W. H., Gregory, L. H., Heft, H. L., Lancefield, D. E., MacLeod, G., Morgan, T. H., Packard, C., Richards, H. M., Sherman, H. C., Sturtevant, A. H., Rose, M. S., Wilson, E. B., Wood, F. C.

Cornell University Medical College.—Bagg, H. J., Bailey, H., Benedict, S. R., Cattell, M., Cecil, R. L., Chambers, R., Chambers, W. H., Churchman, J. W., Coca, Arthur F., Deuel, H. J. Jr., Du Bois, E. F., Edwards, D. J., Elser, W. J., Ewing, J., Foster, N., Hartwell, J. A., Hatcher, R. A., Kahn, M. C., Lusk, G., Miles, W. L., Page, I. H., Papanicolaou, G. N., Reznikoff, P., Richardson, H. B., Schloss, O. M., Stillman, R. G., Stockard, C. R., Thro, W. C., Torrey, H. B., Torrey, J. C.

Harriman Research Laboratory, Roosevelt Hospital—Falk, K. G., Greenwald, L., Lyle, W. G., Sharlit, H.

Johns Hopkins University—Abel, J. J., Amoss, H. L., Brown, J. H., Bull, G., Carter, E. P., Davison, W. C., Dunn, H. L., Harrop, G. A., Jr., Hill, E. C., Holt, L. E., Jr., Howell, W. H., Howland, J., Jennings, H. S., Lee, F. C., Longcope, W. T., McCollum, E. V., Macht, D. I., Marshall, E. K., Jr., Meyer, A., Meyer, A. L., Pearl, R., Perlzweig, W. A., Pfeiffer, J. A., Welch, W. H., Wislocki, G. B.

Montefiore Hospital—Baumann, E. J., Brand, E., Levine, M., Marine, D., Ringer, A. I., Ringer, M., Sandberg, M.

Mt. Sinai Hospital—Baehr, G., Crohn, B. B., Elsberg, C. A., Epstein, A. A., Ottenberg, R., Rothschild, M. A., Schick, B.

New York Homeopathic Medical College—Danzer, C. S., Kleiner, L. S., Stark, M. B., Youland, W. E.

New York University and Bellevue Medical College—Coombs, H. C., Barber, W. H., Brooks, H., Coleman, W., De Graff, A. C., Eisberg, H. B., Gettler, A. O., Hess, A. F., Jackson, H. C., Krumweide, C., Mandel, A. J., Noble, W. C., Jr., Park, W. H., Pellini, E. J., Ratner, B., Prewitt, R. V., Semon, H. D., Symmers, D., Wallace, G. B., Wible, C. L., Zingher, A., Mandel, J. A.

New York Post-Graduate Medical School—Bailey, C. V., Cohen, M., Chace, A. F., Crampton, C. W., Halsey, R. H., Kast, L., Killian, J. A., MacNeal, W. J., Mosenthal, H. P., Marshall, C., Jr.

College of Physicians and Surgeons, Columbia University—Bailey, C. H., Bauman, L., Draper, G., Friedman, G. A., Gay, F. P., Gies, W. J., Harrow, B., Hopkins, J. G., Jobling, J. W., La Mer, V. K., Lee, F. S., Lieb, C. C., McKinley, E. B., Oppenheimer, B. S., Ornstein, G. G., Pappenheimer, A. M., Myers, C. N., Parker, J. T., Penfield, W. G., Pike, F. H., Scott, E. L., Sittenfield, M. J., Thomas, A. W., Vogel, K., Weiss, C., Weiss, H., Williams, H. B., Zucker, T. F.

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